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METABOLIC RATE RELATED TO BODY COMPOSITION IN LEAN MUSCULAR HUMANS

by

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S U M M A R Y

The aims of the present study were: i) to analyse the relationship between the basal metabolic rate (BMR) and the energy ependiture (E.E.) during a standardized physical activity (treadmill walking metabolic rate, TWMR) in a sample of 157 young, healthy, lean and lean and muscular females and males; ii) to analyse the relationship between BMR and TWMR with body mass (B.M.), fat free mass (FFM) and other body composition (B.C.) variables; and, in view of the results obtained, iii) to critically examine the basic assumptions of some of the most widely used methods to assess B.C. For the average population, the use of B.M. or FFM to predict BMR give similar results, but the question arises whether this is the case on individuals on the extremes of the B.C. range such as the very obese or the very lean or lean and muscular subjects.

BMR varied widely in both sexes (CV 12%) and in women it was slightly better explained by differences in FFM; although the slope was similar for both sexes, the constant term differed significantly. While FFM tended to eliminate differences in BMR between sexes, differences between methods in estimation of FFM were important.

Except when analysed by power function ratio standard, BMR per kg FFM decreased as FFM raised; heavier individuals are more muscular and, according to the literature, their BMR/kg FFM decreases because of the low metabolic rate of muscle. Total body potassium (TBK) -an indirect index of muscularity- explained this decrease in males.

In women (n=28) inclusion of TBK improved BMR prediction by B.M. in 10% and BMR prediction by FFM in 2%; in males (n=38), inclusion of TBK improved BMR prediction by B.M. in 5% but when age was included, TBK was not further significant.

In our sample, BMR prediction by the FAO/WHO/UNU, 1985 equations underestimated measured BMR, slightly in females and

significantly in males (mean difference 1.7 ± 9.58 and 5.6 ± 9.41 kcal/day, respectively). In males, Dubois and Dubois and Cunninham's predictions of BMR were not significantly different from measured BMR.

TWMR was 3.2 * BMR for females and 3.6 * BMR for males; these factors slightly differed from those of FAO/WHO/UNU, 1985.

The variable that best explained TWMR variance in females was BMR; for males, the variables that best explained TWMR were B.M. by linear regression analysis (r^2=0.64) or by power function ratio standard models and FFM by linear regression analysis (RSD = 0.32 kcal/min, in the 3 cases). By stepwise regression analysis the RSD slightly decreased when all B.C. variables were included. TBK added nothing to TWMR prediction but -as indicator to muscularity- it partially explained the decrease in TWMR per kg FFM from light to heavy males.

Fat and FFM were estimated in the 157 subjects by body density (underwater weighing, UWW, and skinfolds, SkF, using the equations of Durnin and Womersley, 1974), TBK was measured only in 27 females and 38 males. The Index of Concordance showed lack of agreement among the 3 methods except for the comparison between UWW and TBK in males. A trend to larger differences in leaner and more muscular subjects was found suggesting that either the assumed FFM density (1.1 g/cm^3) or its assumed K content (60 and 68 mmol/kg for females and males, respectively) or both are not wholly valid for all individuals.

The mean amount of K per kg FFM (FFM obtained by UWW) under the assumption of a FFM density of 1.1g/cm^3, were 64 (57 to 72) and 69 (60 to 75) mmol/kg FFM for females and males, respectively. Using these instead of the assumed K/FFM values, a better between-methods agreement in mean fat% and FFM was obtained.

K content and density of FFM did not differ significantly between subjects classified by intensity and type of customary

physical activity. The use of the factors proposed by Womersley et al, 1976 for different intensities of physical activity was not advantageous over the use of constant figures for either sex.

Evidence supporting a constant density of FFM is given; K/FFM was found not to differ between sexes but depended on the amount of FFM.

Probably, the most important finding of this study was that the use of equations, instead of constant figures, to predict FFM and fat% from TBK measurements is a better option.

Since SkF tended to underestimate FFM by UWW in most subjects of this sample, new equations relating SKF to Density were developed for lean muscular subjects. As for the Durnin and Womersley, 1974 equations for subjects of average body build, the log of the sum of 4 SkF was chosen as the best predictor of body density for both sexes.

As it is discussed, the mineral component would not explain the different relationships of SkF to body density between this and Durnin and Womersley studies and between sexes. Rather the different fat distributions between sexes, the lack of sites selected to measure SkF (at the lower limbs) and differences in the internal/external fat relationship between the sample of both studies may be the reasons but a shift in the density of the FFM in lean-muscular subjects cannot be ruled out.

At the light of the results of this thesis, estimations of E.E. and B.C. in lean muscular subjects would require somewhat different factors than those used for the average population although the predictor variables are the same.

Needless to say, estimations of E.E. and B.C. through equations are mere approximations for groups of subjects; the biological variability is so wide that accurate values for individuals can only be achieved by direct measurements under strictly controlled conditions.

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Abbreviations

AMA = arm muscle area
App. = appendix
ATFM = adipose tissue free mass
ANOVA = analysis of variance
B.C. = body composition
B.D. = body density
B.M. = body mass
B.t° = body temperature
B.C.S. = Brussels cadaver study
BMR = basal metabolic rate
BSA = body surface area
CV = coefficient of variation
D = densitometry
E = energy
E.E. = energy expenditure
E.R. = energy requirements
F = atmospheric correction factor
FFM = fat free mass
LBM = lean body mass
LRA = linear regression standard
M.R. = metabolic rate
N = nitrogen
PFRSt = power function ratio standard
R.Q. = respiratory quotient
Res. Vol. = residual volume
RSD = residual standard deviation
SD = standard deviation
S.G. = specific gravity
SkF = skinfolds
SRS = simple ratio standard
STDP = standard temperature and pressure, dry
Σ3crc = sum of 3 circumferences (arm, thigh, calf)
t° = temperature
TBK = total body potassium
TWMR = treadmill walking metabolic rate
UWW = under water weighing
V = volume

DECLARATION

I declare that the work presented in this thesis was carried out by me and it does not include work forming part of a thesis presented for a degree in this or any other University.

Signature: Ma. Teresa Espinosa Z. date 1st May, 1995

CHAPTER 1

GENERAL BACKGROUND

Many applied nutrition activities -evaluation of nutrient intake data, nutrition education, planning of food supply systems, etc. - depend on recommended nutrient intake values which are figures statistically derived from actual requirements measured in individuals grouped by sex, age and other pertinent characteristics. Due to their statistical nature, recommended nutrient intakes are implicitly collective figures to be used for collective purposes. While for most nutrients the recommended value represent the mean plus two standard deviations of the available data on individual requirements, the recommended value for energy usually represents the mean of the requirements.

Recommended intakes are proposed and periodically reviewed by groups of experts at the national or at the international levels. The best known and most widely used international recommended values are those of the United Nations agencies. In its last report (1985) the joint FAO/WHO/UNU Committee utilized a new approach to calculate energy requirements (E.R.) based on the measurement of total energy expenditure (E.E.). E.E. may be broken down into different components among which the most important are the basal metabolic rate (BMR) and the expenditure on physical activity. Perhaps the main innovation of the Committee's approach was to express the components of E.E. as multiples of BMR as this is usually the largest and most predictable of the E.E. components. Such strategy emphasizes the need to estimate BMR as accurate as possible as any over or under estimation of its value would significantly affect the overall calculation of E.E..

The measurement of BMR is cumbersome and requires strict standardization and care. From decades of experience on BMR measurements enough data has been gathered to allow for predicting BMR from variables more easily measured or identified such as sex, age and body mass. A great number of predictive equations have been proposed in this regard; sex and age are body mass independent factors and body mass is the most practical predictive variable.

Since BMR per kg of body mass is not constant over the range of body masses tending to decrease in heavier individuals other predictive variables have been proposed. Fat free mass (FFM) would theoretically be a good predictor of BMR since its use eliminates the variability by sex and age and since a given body mass may vary in composition. Several equations based on FFM have been proposed, outstandingly Cunninham's, but body mass is still the most widely used predictor of BMR and it is used in numerous equations. The FAO/WHO/UNU, 1985 report proposes several equations for different age periods and for the two sexes which are entirely based on body mass. Nevertheless, the combination of age, sex and body mass cannot

explain all the variability in BMR prediction; height does not help to decrease that variability.

On theoretical bases the differences in body composition among individuals with the same body mass would affect the prediction of BMR but generally this effect is considered to be very small. Notwithstanding, on the extremes of body composition this factor may have a more pronounced effect than generally recognized for non extreme body composition differences; therefore, the relation of BMR to different body components in individuals with extreme deviations (i.e., extremely lean or extremely fat) in body composition deserves study.

FFM is the body component which includes muscle, skeleton, skin, viscera, blood and other minor components. FFM may be estimated through different techniques such as measurements of body potassium, skinfolds and other anthropometrical parameters, underwater weighing, total body water content and other newer techniques which however are not usually available under most conditions.

The estimation of FFM is limited by the implicit suppositions underlying each technique. For example, underwater weighing and the skinfolds approach assume a relative constancy of the density of FFM and fat mass, the body potassium and total body water techniques assume a rather constant potassium and water content in FFM and the anthropometric technique assumes that fat is uniformly distributed in the body and that the ratio internal to external fat is fixed. Since repeated measurements have shown that in fact the variability of those physiological parameters is very small in the average population, the above assumptions are considered valid and safe for that population. Again, this may well not be the case for those groups in the extremes of the body composition spectrum such as the very obese, the very lean and particularly the very lean and very muscular individuals. For example, the previously mentioned decrease in BMR per kg of body mass from light to heavy individuals could possibly be explained by a larger muscle mass in subjects with large body mass; since muscle is proportionally richer in potassium, the subjects may falsely appear as having a larger FFM while they actually may have a FFM with higher than assumed potassium content.

Very lean and specially very lean and muscular subjects are usually physically very active. Muscle mass hypertrophy affects the assumed composition of FFM since muscle is richer in fat, potassium and water and has a lower density than other FFM components. Physical activity may also produce a higher mineralization of the skeleton and a higher density of this FFM component. Thus the lean and muscular subjects may be subjected to a body density lowering factor (high muscle mass) and to a body density rising factor (more mineralization

of bones) these opposing factors may or may not cancel each other therefore altering or not the interpretation of the FFM estimation; this needs to be clarified. Does the opposite occur in very obese subjects?.

In any case, the study of the very lean and muscular subjects should throw light on those questions and allow to decide whether body mass is or is not an adequate BMR predictor. Any tendency observed as subjects become leaner and more muscular would be important to take into account in the definition of strategies, parameters and interpretation criteria. Should the basic assumptions of the techniques not hold for lean and muscular groups new assumptions and factors would need to be determined.

The present study intends to critically examine the validity of the basic assumptions of some of the techniques more widely used for estimating body composition in the two components model.

CHAPTER 2

BASAL AND EXERCISE METABOLIC RATE

LITERATURE REVIEW.

The factors contributing to the total energy requirement (E.R.) are ultimately determined by the internal and external work of the body. The determination of E.R. requires a disaggregation of the total energy expenditure (E.E.) into physiological entities that can be defined and measured. The most significant factors that affect the total E.R. of an individual are: basal metabolism, food thermogenesis and physical activity.

This study deals on the E.E. under basal conditions as well as while walking on a treadmill under controlled- standardized circumstances.

1. BASAL METABOLIC RATE (BMR).

BMR refers to the metabolic activity required by the body for maintenance of normal functions and homeostasis under complete resting and standardized conditions. In order to minimize influences that would raise metabolic activity and invalidate baseline comparison among individuals, the extent of cellular metabolism must be determined under closely controlled and standardized conditions. The criteria to be met for a measurement to be considered basal are: the subject should be lying awake in a state of complete physical repose, free of muscle tension, at least 12-14 hours after the last meal and/or vigorous physical activity; the environment should be thermoneutral and comfortable, in the absence of emotional disturbance, fever and disease (Benedict, 1915; Boothby and Sandiford, 1929; Boothby et al, 1936).

BMR does not refer to the absolute minimum level of E.E. compatible with life as it has been shown to fall below 'basal' during sleep (Passmore & Durnin, 1967); but in practice it is considered to be approximately equal to E.E. of subjects during sleep (FAO/WHO/UNU, 1985). It is a convenient base from which to evaluate additional costs.

BMR varies with sex, age, environmental temperature, nutritional status, and other factors that are discussed below. Identification of specific metabolic components is valuable in understanding the nature of these variations. Oxidation of nutrients in the body provides ATP which is utilized to perform the internal work. While exact figures cannot be given, some estimations have been done by Baldwin & Smith, 1974. **"Service" functions** derive up to about (≈) 45% of basal heat production and include sodium transport by the kidney (≈7%), blood circulation (≈11%), respiration (≈7%) and nervous functions (≈20%). **Cellular maintenance functions** include costs of resynthesis of labile tissue proteins and triglycerides (≈10%) (a typical example is that of protein turnover; also included are the

substrate or 'futile' cycles which exist at certain points in intermediate metabolism) and ion transport for the maintenance of membrane potentials; for example, sodium and other ion pumps and all processes of active transport (≈20-30%). Reeds et al, 1985 have estimated the contribution of protein turnover to BMR to be somewhere in the order of 11-15% and the contribution of the energy costs of substrate cycles to basal expenditure to be about 15%.

For the majority of subjects, BMR represents a large proportion of the total E.R., this proportion decreasing as physical activity increases. Given that BMR may represent from half up to two thirds of the total E.R., differences in BMR are important determinants of differences in daily energy needs.

1.1. Factors that have an influence on the variability of the BMR. Numerous determinations of BMR have been made on humans and other species and it is clear that metabolic rate (M.R.) varies with **body size**; investigators have made attempts to take this into account when making comparative measurements. There has however, been discussion and disagreement in attempts to establish a constant relationship between M.R. and a unit of body size that would apply to large and small animals alike (Kleiber, 1947).

For many years it was customary to express BMR in relation to body surface area (BSA), that is kcal per hour per square meter of body surface (kcal/hr/m^2). This relationship is based on the assumption that heat production and therefore BMR are proportional to BSA which, in humans can adequately be estimated by the formula of Dubois and Dubois, 1916.

From a comparison of data on M.Rs. of several mature mammalian species, Kleiber, 1932, 1947 found that a more precise, and applicable to all species, relationship than BMR and BSA is that of BMR and the three fourths power of body mass (B.M.$^{3/4}$). He found this relation by plotting the logarithms of BMR and body mass (B.M.) and as they were linearly related he concluded that BMR was directly proportional to B.M. raised to 0.75. The significant advantage of this equation is the wide range of mature B.Ms. among homothermous over which it is applicable. Kleiber analysed data of Harris and Benedict, 1919 to develop equations for M.Rs. in women and men adjusted for age and stature.

Some investigators, as Heusner, 1985 among others, have criticized the use of BSA and B.M.$^{0.75}$ in humans for not being properly justified.

Age is another factor affecting BMR and its variation throughout the life span. BMR is highest during periods of rapid

growth, associated with the increased biosynthetic activity of growth (Spady et al, 1976). However, differences in BMR at different stages of life may also obey to differences in body composition (B.C.), specially in FFM. Once maturity is reached BMR tends to fall. According to FAO/WHO/UNU, before the age of 60 years the fall is comparatively small, about 1-2% per decade, but becomes more pronounced thereafter. The reason for age changes in BMR is not well understood.

The **sex** difference in BMR noted at almost all stages of life, which appears to represent between 6 and 10% from the age of 5 years into old age (Boothby et al, 1936), often is attributed to differences in B.C. between males and females. It is thought to be largely attributable to the fact that the female body contains more fat and less muscle than men at a given B.M., but these different proportions do not explain the difference in BMR between the sexes. According to Kleiber, 1961 and Mitchell, 1962, the effect of the sex hormones on BMR may be more direct than their effect on B.C..

Racial differences apparently do not affect BMR and the effect of environmental temperature (t°) is uncertain. The results of some studies have suggested that in relation to B.M., Asian subjects have lower BMRs than their North American or North European counterparts (Schofield, 1985; McNeil et al, 1987, Drummond, 1988; Hayter & Henry, 1994). It is not known whether this is a result of genuine genetic differences between racial groups or relates to differences in nutritional status, diet, climate, or B.C.. Lawrence et al, 1988 have shown that differences in the BMRs of Scottish, Gambian and Thai women could be explained by the amount of fat free tissue in the body and found race, climate and nutritional status to have little effect.

Climate. Although some reports indicate that individuals living in tropical climates tend to have lower BMRs than those living in cold climates, Consolazio et al, 1961 have reported no effect of excessively hot climates on the BMR (Pike & Brown, 1975), the results are conflicting.

Within the thermoneutral range, 22-30°C for humans, it is usually believed that no adjustment of BMR is necessary to maintain a normal body temperature (B.t°) of 36-38°C; however, Dauncey, 1981 found a 6% rise in M.R. with a fall in environmental room t° from 28°C to 22°C. Above the upper body critical t°, heat loss cannot keep pace with body heat production, and M.R. increases 12% for each 1°C rise in B.t°; but above a 'critical' t° an increase in B.t° happens and the body dies from heat. M.R. increases as environmental t°

decreases until it reaches a maximal (summit metabolism). A further decline in environmental t° cannot be compensated for, and B.t° falls and the body dies from cold (Brody, 1945). Attempts to identify adaptive increases in BMRs of individuals living in different climates have produced conflicting results. Buskirk et al, 1957 found no significant difference in BMR among three groups of soldiers studied for at least one month. Mean ambient t° differed among the three locations by 59°C, (from -25 to 34°C). Diet composition and energy (E) intake were the same for all groups. Gold et al, 1969 found seasonal differences in M.R.. Consumption of O_2 was lower in the summer during periods of rest and exercise. Diet was not controlled on this group. On the other hand, Mason and Jacob, 1972 reported data from individuals whose BMRs were altered by a change from a tropical to a t° climate or vice versa. Recent FAO/WHO Committees on E.Rs. have not made an allowance for climatic factors in predictions of BMR, but concede that further investigation is required before it can be concluded for certain that t° and humidity have no important effect on BMR (FAO/WHO/UNU, 1985).

Nutritional status. **Overnutrition** relation to BMR is an issue of dispute. Chronic overfeeding leads to an increase in B.M., consequently both fat and FFM increase and concomitantly BMR increases as a result. However, over-eating does not always produce a proportionate gain in body energy; Sims, 1976 and Schutz et al, 1982 have postulated that over-feeding stimulates an increase in BMR over and above that resulting from changes in B.C. The concept of an increase in E.E. in response to excess E intake, termed "luxus konsumption" (term induced by Neumann, 1902 to explain his apparent ability to maintain B.M. on two different levels of E intake, by a mechanism that converts excess E intakes directly into heat), is supported by results of studies of humans which, although not conclusive, are difficult to explain by other means. It has also been suggested that this occurs through hormonally-induced alterations in the metabolic activity of the tissues (Crist et al, 1980).

Norgan & Durnin, 1980 performed a very careful constructed and extensive overfeeding experiment in which moderate weight gains were achieved, they found that M.Rs. in standard tasks were 10% higher at the end of overfeeding, but expressed as units of E per kg per minute were similar to control values. Mean E gain was less than excess E intake and the authors discuss that such a discrepancy is unlikely to be due to unmeasured increases in M.R. but could have arisen from errors in the calculation of the variables involved. Their conclusion is that increases in M.R. appear to be associated with increased body size and tissue gain rather than a luxus-konsumption mechanism.

Effects of **undernutrition** on BMR are more clearly defined. BMR reduction by undernutrition may result from a decrease in tissue mass or a lower activity per unit of tissue. The contribution of each effect is dependant upon the duration of food restriction. Short-term restriction to 1000 kcal/day for 13 or 19 days produced a decline of 17-21% in BMR regardless of the basis of comparison (Grande et al, 1958), lost B.M. and M.R. returned rapidly to normal with refeeding. The initial fall may occur without greater losses in FFM. Many authors however, have also reported a decrease in BMR over and above that expected from tissue loss alone (Grande et al, 1958; James et al, 1978; Bessard et al, 1983; Finer et al, 1986; Barrows & Snook, 1978). Two explanations for this reduction have been offered:
1) there may be an alteration in the composition of the lean tissue mass. During E restriction tissues with high M.Rs., such as the liver, are initially lost at a proportionally greater rate than other less active tissues (Grande et al, 1958). If this were the case a fall in the overall M.R. of the fat free tissue would result.
2) the metabolic activity of the individual tissues themselves fall (James et al, 1978). The decrease in thyroid hormones and catecholamines levels associated with E restriction have been suggested to bring about such a change (Jung et al, 1980; Shetty et al, 1979).

The above hypotheses are not mutually exclusive and it may well be that the decrease in BMR per unit of weight of tissue is the result of a combination of them both, depending on the duration of food restriction.

There has also been found some influence of the performance of **physical activity** on BMR. Many attempts have been made to show that BMR is higher for athletes than non-athletes due presumably to a greater FFM (Mitchell, 1962). Slight increases have been shown in the trained athlete but differences between athletes and non-athletes generally appear to be negligible (Pike & Brown, 1975).

Body Composition (B.C.). The degree of body fatness will affect BMR, such that at a given B.M. the greater the fat content the lower the M.R.. Besides this fact, it has been suggested that the variability which is observed when comparing individuals of different sex, age, ethnicity, physical activity and B.M. disappears when values are expressed per unit of FFM (Webb, 1981; Jequeir, 1987).

Sex difference in BMR has been found by several researchers to be largely eliminated once FFM has been taken into account (Cunninham, 1980; Bernstein et al, 1983; Ravussin et al, 1986; Owen et al, 1987). Athletic training has also been found to eliminate

differences between sexes even when BMR is expressed per unit of B.M., because fat mass and FFM become more similar in athletes. This fact lends support to the idea that there are not any inherent differences in the metabolic activity of the tissues themselves between women and men (Cunningham, 1982). However, at least the menstrual cycle makes sexes to differ among them (Bisdee et al, 1989).

Some studies have reported aging effect on reducing BMR to be not significant once FFM differences have been taken into account. The reason is that differences obey to changes in the FFM (Keys et al, 1973; Cunninham, 1980; Ravussin et al, 1986; Owen et al, 1987). However, other studies have found that BMR or resting M.R. diminished with aging even after adjusting for differences in FFM (Mc Neill et al, 1987; Doré et al, 1982).

Lawrence et al, 1988 showed that differences in BMR between women from Scotland, Thailand and Gambia, were largely eliminated once FFM had been taken into account.

The comparison of individuals with different B.M., taking into account the effect of fatness on M.R., has found no significant difference in the relation between BMR and FFM in lean and obese subjects; that is, individuals with the same FFM have the same BMR no matter what their body fatness is (James et al, 1978; Doré et al, 1982; Ravussin et al, 1982; Lawrence et al, 1988).

Much of the variation in BMR between groups of different sex, age, B.M. and ethnicity may be largely reduced once FFM is taken into account and it could serve as a useful reference standard. However, even when FFM differences have been accounted for, great differences, i.e., 30-50% have still been found between individuals of relatively homogeneous (age, sex, race) groups (Bernstein, 1983; Jequier, 1987; Lawrence, 1988). Moreover, in such groups BMR relates almost equally either to B.M. or FFM. The source of variability is not easily identified (Woo et al, 1985) and could suggest the control of yet unidentified factors controlling BMR. In situations such as this when the use of either variable, B.M. or FFM, predict almost equally BMR, the use of B.M. is preferable because of the ease of this measurement.

At a given FFM, between subject BMR variability has been found. Durnin et al, 1985a did a comparison between men of similar FFM and found a S.D. of about 10% in the BMR.

Residual standard deviation (RSD), obtained from regression analysis between BMR and FFM, also give some idea of the variability; Lawrence et al, 1988 obtained RSDs between 100-150 kcal/day for the 3 groups of women studied.

The use of FFM to compare BMR of individuals leaves still a lot of variation to be explained; the source of variability is not easily identified (Woo et al, 1985). Intra-individual variation, problems in the estimation of FFM, the variability of the composition of the FFM, differences in the metabolic activity of the tissues and the differences in the basic E demanding processes at cellular level, all combined may be the cause of such variations.

Intra-individual or within subject variability in BMR may arise from differences in the preceding day's E intake, level of exercise, the stage of the menstrual cycle for women and errors in the measurement of E.E.. The combination of all these factors have been reported not to be of great importance compared to inter-individual variation.

The problems related to FFM prediction mainly arise from the assumptions employed by either of the methods employed for this purpose and the measurement error of each method. A detailed description on this matter is presented in the chapter on B.C., sections 3.1.3.- 3.1.8.

The composition of the FFM is not constant and the variation of each component is, unfortunately, not precisely known as methodological errors are involved and the number of cadavers studied is scarce. For the purpose of B.C. estimation it is assumed to be of 'fairly' constant composition; however, from the point of view of the metabolic activity of each component those variations may be important. The liver, brain, heart, and kidney account for around 60% of the resting oxygen consumption and they represent only about 6% of the FFM in terms of mass (Brozek and Grande, 1955). Skeletal muscle may comprise from 40-50% of the FFM and it contributes to less than 20% of BMR (figure 2.1).

Organ	Mass[kg]; %	BMR [kcal/day]	% of whole body BMR
Liver	1.60; 2.3%	482	27
Brain	1.40; 2%	338	19
Heart	0.32; 0.5%	122	7
Kidney	0.29; 0.4%	187	10
Muscle	30.0; 42.9%	324	18
Remainder	36.4; 51.9%	-	19
Total	70; 100%	1800	

Figure 2.1. Contribution of organ and tissue metabolic rate to BMR in man.
Source: FAO/WHO/UNU, 1985.

From this figure it can be seen that FFM is made up of tissues of very different relative proportions and M.Rs.; then, differences in the composition of the FFM may influence BMR. Lawrence et al, 1988 have suggested that systematic differences in the composition of the FFM may provide an explanation for the finding that BMR/FFM [kcal/kg] tends to be lower in heavier compared to lighter individuals. This could be the case if as B.M. increased, the proportion of the FFM occupied by metabolically active organs declined and concurrently the proportion of relatively inactive tissue such as muscle increased.

1.2. Use of BMR as part of equations to predict energy requirements.
The Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements, 1985 defined E.R. as the amount needed to maintain health, growth and an appropriate level of physical activity. The report of the Joint provides information on which judgement of health and appropriate level of physical activity can be based. Three general concepts established by FAO Committees on E.R. are given to best determine E needs:

a) the E need of a group is represented by the average of the needs of individuals in that group.

b) As far as possible, E.R. should be determined from estimates of E.E.

c) The E.R. of a "reference" man or woman constitutes the baseline of people in general. Adjustments are then made for deviations from those reference requirements for different states and situations such as growth, pregnancy, lactation, aging, climate, etc.

As in the great majority of cases the largest component or a substantial proportion of total E.R. is accounted for by the BMR, the Consultation adopted the principle of calculating all components of E.E. as multiples of the BMR. Although they recognized that this principle, used for the sake of simplicity, is likely to involve some inconsistencies because of the known factors to affect BMR.

The Committee determined that for practical purposes the most useful index of BMR is the B.M., the data base for developing equations covered some 11 000 technically accepted measurements of individuals of both sexes and all ages, who were considered to be healthy. In the opinion of the Consultation, these equations can be regarded as the best estimates available to 1985 for predicting the BMR of healthy people in any population. But direct measurements are to be preferred when these can be made.

The calculation of E.R. proceeds in two steps:

a) The BMR per day is determined from the regression equations from either actual or desirable B.M.. When the E need per kg is required, it can be derived by dividing the calculated BMR by the B.M.. The Consultation recognized that even at a fixed age, the BMR per unit B.M. is not constant for all weights, this has been taken into account and the effect is to increase the estimated requirement of smaller and lighter people and to decrease the requirement of those who are larger and heavier. Age is also considered, adults of both sexes are divided into three age ranges: 18-30, 30-60, 60+.
b) To obtain the total E.R., the estimate of BMR is multiplied by a factor that covers the E cost of increased muscle tone, physical activity, the thermic effect of food, and, where relevant, the E.R. for growth and lactation.

1.3. Factors given by FAO/WHO/UNU to predict total energy expenditure.

a) Baseline E need: Since BMR is measured in the post-absorptive state and complete rest, for an individual to survive an addition has to be made to cover the metabolic response to food and the E cost of increased muscle tone and minor movement. A value of 1.4 times the BMR during waking hours, for the E cost of personal activities has been derived. The BMR is the key to the expression of E.Rs. because all other E costs are considered as multiples of the BMR. Only a small error is introduced with including the thermic response to food. The dietary component varies a little with the type of food eaten, but it is predictable for a group of subjects.

b) Energy needs for occupational activities. The E need will vary with the type of occupation, the time spent in doing the task, and the size of the individuals concerned. The estimates of the requirements per minute for various occupations are given in the report and there exist other sources (James and Schofield, 1990). These are expressed as multiples of the BMR, and thus include the minor movement, muscle tone, and the specific metabolic response to food.

Depending on the required accuracy of the E.R. different stages of simplification to estimate the E.E. for groups of individuals have been offered.

The actual E.E. can be assessed either measuring the subjects' expenditure in a metabolic ward while performing simulated activities for the period or had all their activities monitored with measurements of the E cost of each activity.

The estimation of E.E. may be obtained in three stages of simplification:

Stage 1: detailed activity monitoring. This stage dispenses with the continuous monitoring of E.E.. If the time allocated to different activities is known, then the actual costs of these activities can be taken from tables which summarize estimates of the E cost of each activity and calculations of minute by minute E.E. can be assessed.

The activity pattern analysis must be accurately described and the time spent on each activity preferably monitored by a trained observer. The approach may be simplified by specifying the activities performed in each 15-min period or an even simpler approach, recording the approximate time in hours spent on specific tasks. As the monitoring becomes cruder it is more difficult to be certain of the validity of the estimations of E.E. because few people at work are continuously active, and the number of pauses during an activity can make a substantial difference to the final E cost of the work.

Physical activity ratios (PAR) may be obtained by dividing the E cost of individual activities maintained on a min-by-min basis and expressed as a ratio of the BMR. The E cost of women and men undertaking the same task has been shown to be very similar once it is expressed as a PAR; also, people with very different B.Ms. and therefore different rates of E.E., have the same activity ratio. Thus, by calculating BMR first, an E cost ratio of the activities performed by a group of individuals of different sex and B.M. can be assigned. The PARs of the individual activities performed by a given subject have to be collated to obtain a reasonable estimate of the overall average rate of her/his 24-h E.E..

Stage 2: Average activity estimates for periods of the day. As the above method may be too time consuming for population studies, this method is recommended for government planners.

In this stage, the activity pattern analysis is assessed by grouping the activities into four categories which can be built up to cover the 24-h period. The categories are:

1. Time spent in bed: the overall E cost is taken to be the same as the BMR;
2. Occupational time (O): E cost estimates are chosen appropriate to the task;
3. Discretionary activities: Household tasks (H), socially desirable activities and activity for physical fitness and the promotion of health. E cost estimates are chosen for the overall type of activity;
4. Residual time (R): when individuals are not engaged in major E consuming activities: the E cost is designated as 1.4 the BMR.

Even though the rate of E use during sleep is about 95% of the BMR, its use has only a negligible effect on the estimates of E.R..

The pauses in work during occupational time are integrated to values that can be used for different occupations. Traditionally the occupations of women & men have been classified into those which involve light, moderate, and heavy physical activity. This has facilitated the broad assessment of the E.Rs. of populations and has been helpful when the E needs of a particular occupation have not been specifically studied; the approximate values for the E costs of occupations involving the three degrees of activity have been obtained. The gross E.E. can be estimated as 1.7, 2.2, and 2.8 times the BMR for young women and 1.7, 2.7, and 3.8 times the BMR for young men at light, moderate and heavy activity levels, respectively.

Discretionary activities may be more difficult to assess but they should be evaluated, since they usually contribute to the physical and intellectual wellbeing of the individual, household, or group.

The E cost of activities are determined giving to each period of activity an Integrated Energy Index (IEI) which is the E cost of the activity or occupation including the pauses for rest expressed on a minute or hourly basis and calculated again as a ratio of the BMR.

The FAO/WHO/UNU report, 1985 used values to illustrate the process of calculation from observed activity patterns described in the literature. Although these values are not proposed for general application, they can be used to derive crude estimates of the average E allowances of a community. The IEI as multiples of BMR of discretionary activities of adults are: Optional household tasks: 2.7; Socially desirable: 3.3; Cardiovascular and muscular maintenance in adults with light activity only: 6.0 from 18-60 and 4.0 for > 60 years. Sometimes the IEI for discretionary activities including socially desirable and household tasks has been integrated as: 3.0.

Stage 3: Single values for the whole 24 hours. On the basis of activity patterns observed, approximate estimates of the total daily E.E. corresponding to light, moderate and heavy work can be derived as multiples of the BMR; this has been done by integrating the discretional activities into the whole's day E needs based on occupation. The values of these activities are termed the physical activity levels (PAL); the integrated value are for light, moderate and heavy activities: 1.56, 1.64, and 1.82 for women and 1.55, 1.78, and 2.10 for men.

It must be emphasized that these figures are intended to be general guidelines, whenever possible calculations should be made as explained for stages one or two above.

2. METABOLIC RATE OF PHYSICAL ACTIVITIES UNDER STANDARDIZED CONDITIONS.

The E cost of physical activities under standardized conditions may be obtained by measuring the oxygen uptake of individuals while performing a given activity, either with a Douglas bag to collect the expired air or a spirometer such as the Kofrani-Michaellis apparatus. Passmore & Durnin, 1967 have used the rates of oxygen consumption, i.e., indirect calorimetry to assess the E.E. while a definite activity is undertaken for a limited period of time, usually measured in minutes.

The different types of activity undertaken by an individual can be identified and the time spent in each activity measured. The E cost of each activity can then be obtained and by adding up the various metabolic costs of these activities, the E expended during a whole day can be estimated. Much information is available to assess the E.E. in daily life. E.E. must be matched with E ingested if health and activity are to be maintained. It has been stated that E.R. should preferable be assessed from E.E. (FAO/WHO/UNU, 1985).

The metabolic costs of the various possible activities should be known in order to be able to assess the E.E. of an individual or group.

The physical activity is considered by the FAO/WHO/UNU Consultation to be composed of occupational and discretional activities. Occupational activities are those essential for the individual and the community, and are considered as economic activities which are life sustaining. "Leisure-time" activities have been termed "discretional", as they are considered to be desirable for the well being of the community and the health of the individual and the population, these activities have been divided into three categories: optional household tasks, socially desirable activities and activity for physical fitness and the promotion of health.

3. MEASUREMENT OF ENERGY EXPENDITURE.

Human E.E. may be measured either by direct or indirect calorimetry. Both have been applied to assess M.R. and short- and long- term assessment of E balance in health and disease states, such as obesity, undernutrition, cancer, trauma and infection among other disease states, and exercise.

As an introduction to the topic of calorimetry, a brief history of bioenergetics, compiled by Buskirk and Mèndez, 1980, is given below.

The science of bioenergetics was perhaps initiated with the studies of Lavoiser (1740-90) who discovered the principles of animal

respiration using an ice calorimeter (an apparatus used to measure the quantity of heat) to study the body heat emanating from guinea pigs by measuring the amount of melted ice and determined that oxygen was utilized by the metabolizing body and that a gas was given off; he compared respiration with a slow oxidation of carbon and measured the amount of the gas (carbon dioxide) given off. He got to know that the intensity of metabolism was dependant upon physical work, environmental temperature and food intake.

Liebig, 1842 further increased the knowledge of respiration showing that carbohydrates, fat and protein were oxidized in the body. von Voit in the 1860's reached new findings in the field of protein metabolism and Rubner (≈1900) demonstrated that the laws of conservation of E apply to the living body.

A brief history of E metabolism instrumentation, also compiled by Buskirk and Méndez, 1980, is given below:

The first indirect calorimeter or respiratory chamber was built by Pettenkofer; he and Voit performed several important experiments in their respiratory chamber in the 1860's. The first closed-circuit respiratory apparatus, for use in experiments of indirect calorimetry, was designed by Regnault and Reiset in 1849. The names of Armsby, Atwater, Benedict, Dubois, Lusk and Murlin were associated with the dynamic phase of direct and indirect calorimetric work from 1890 to 1935. Their studies, along with more of many others, form the basis of much of our present understanding of E metabolism. Other instruments include a gradient-layer (heat-flow) calorimeter for direct calorimetry (Bezinger and Kitzinger, 1949), portable systems for indirect calorimetry (Kofrany and Michaelis, 1941; Liddell, 1963: MÜller and Franz, 1952; Passmore et al, 1952) and physical gas analysers capable of continuously measuring a specific gas concentration in a gas stream, some calorimeters with the advantage of being able to follow with high precision rapid changes in body loss (Bezinger and Kitzinger, 1949, Bezinger et al, 1958).

3.1. Calorimetry.

Calorimetry involves the determination of heat loss of the living body, either directly or indirectly.

Direct Calorimetry. The determination of heat loss by direct calorimetry is theoretically simple but, in practice, cumbersome and expensive. It is performed considering that total heat is dissipated by the body in the form of radiation, convection, conduction and evaporation and also that it is lost via the lungs and skin. The sum of these forms of heat loss is regarded as representing the total heat released by metabolism in the body; the major loss either in the

resting state or while performing work is by radiation and conduction from the body. Important differences may be found in water vaporization between the resting and active states (up to 1500 kcal). Direct calorimetry is based on the fact that when heat is conducted across a layer of thermally conductive material, a difference in temperature exists between the two surfaces of the layer. By interlayering thermocouples above an insulating layer, the calorimeter provided a rapid thermal response, and continuous measurements of heat loss were possible. Incorporation of an air ventilation circuit provided for separation of evaporative thermal loss. A separate breathing circuit made possible separation of pulmonary heat loss (Buskirk & Méndez, 1980). Currently, three types of calorimeters are in use to assess heat loss in man. These are the isothermal calorimeter developed by Atwater & Benedict, 1903, the gradient layer calorimeter by Bezinger & Kitzinger, 1949 and a water cooled garment developed by Webb et al, 1972.

Direct calorimetry has the advantage of being extremely accurate for measurements of E.E. over relatively long periods of time (up to a day or more) without causing much discomfort to the participating individuals. However, because of the body's capacity to store heat E and the consequent delayed response between heat production and heat loss, it is inappropriate for short term measurements of E.E. such as measurements of BMR, the thermic effect of food or exercise; nor is it appropriate for measurements of E.E. in large numbers of free living subjects. Moreover, the equipment is complex and expensive to construct.

Indirect Calorimetry. It is based on the calculation of heat production from gaseous exchange: oxygen (O_2) consumed and carbon dioxide (CO_2) expired, or both. If it is assumed that all the O_2 consumed by an individual is used to oxidize degradable fuels and that the CO_2 so liberated is recovered, it is possible to calculate the total amount of E produced. When the rate of nitrogen (N_2) excretion is also known, the type and rate of fuel utilization can also be deduced.

Indirect or respiratory calorimetry is based on the principle that during oxidation of organic molecules in the body, O_2 is consumed in amounts related to the E or heat liberated. For each litre of O_2 consumed there is a known amount of heat which is being liberated by the body. Nevertheless, the amount of heat liberated per litre of O_2 varies depending on the proportions of carbohydrate, fat and protein being oxidized. The heat equivalent of respiratory exchange is not only calculated from O_2 consumed and CO_2 expired but also is dependant upon the molar ratio of CO_2 produced to O_2 consumed, known as respiratory quotient (R.Q.) which varies because

of differences in composition of the foodstuffs that determine the amount of O_2 required for complete oxidation and, consequently, the volume of CO_2 that is given off. For carbohydrate the R.Q. is 1.0 since in combustion of carbohydrate the amount of molecular O_2 required for oxidation is equal to the CO_2 produced. Fats require more O_2 than carbohydrates for combustion because the fat molecule contains a low ratio of O_2 to carbon and hydrogen. Calculation of the R.Q. for protein is more complicated than for fat or carbohydrate because protein is not completely oxidized and both carbon and O_2 are excreted in the urine chiefly as urea. When adjustment is made for urinary excretion, the ratio of CO_2 produced to O_2 consumed is approximately 1:1.2 and thus is equivalent to an R.Q. of 0.80.

Since the nature of fuel consumed in cellular respiratory precesses determines both O_2 consumption and CO_2 formation, the caloric equivalent for a given volume of O_2 or CO_2 also will vary with the R.Q.; nomograms for the caloric values for O_2 and CO_2 for nonprotein R.Q. are available.

For work requiring great accuracy, the extent of protein oxidation may be calculated from urinary N_2, and the nonprotein R.Q. then may be estimated. In practice, the error incurred by ignoring protein metabolism is relatively small and, particularly in short term studies, no correction is made for the effect of protein metabolism on R.Q. Calculation of heat production is made as if only fat and carbohydrate were oxidized (Weir, 1949; Pike & Brown, 1975).

Indirect calorimetry has a short response time due to the body's inability to store O_2 and since anaerobic production of ATP is limited. Because of its flexibility, versatility and short response time, indirect calorimetry is widely used to assess the acute effects on M.R. of stimuli such as food or exercise and for measurement of BMR.

The respiration calorimeter, a chamber somewhat similar to that used in direct calorimetry, was the first instrument to be used for the measurement of respiratory exchange. Thereafter, mobile lightweight and more versatile instruments have been devised.

Indirect calorimetry techniques fall into one of two categories: open or closed circuit.

In the closed circuit indirect calorimetry, subjects are asked to breath through a closed system containing pure O_2. The expired air is passed through soda lime where CO_2 is removed and the remaining O_2 returns to the system. The decrease in the volume of O_2 over a set time gives a measure of O_2 consumption. By using appropriate conversion factors, M.R. (kcal/min or kJ/min) of the individual may be calculated.

In open circuit indirect calorimetry, subjects are asked to breath normal atmospheric air and the expired gases are collected and analysed for O_2 and CO_2 content using specially designed gas analysers. Development of the computerized systems for measuring O_2 consumption made it possible to have an on-line gas analysis, for example in the ventilated hood system or the respiratory chamber. Several open-circuit methods are available; they range from sophisticated respiration chambers suitable for measurement of energy expenditure over several days, to the simple Douglas bag system (Douglas, 1911) which is light, portable and inexpensive, being in many situations the method of choice in normal healthy adult individuals; it may not be used in patients or young children (Segal, 1987).

The respiration chamber is an air tight room which forms an open circuit ventilated indirect calorimetry. Outside air is continuously drawn into the chamber and the flow rate of air at the outlet is measured using a pneumotachograph with a differential manometer. A fraction of the extracted air is continuously analysed for O_2 and CO_2 concentrations. In respiration chambers subjects have room to sleep, eat or exercise; therefore, it is possible to measure E.E. over long periods of time (up to few days) without causing discomfort to the subject. The respiration chamber is considered to be the most accurate open circuit indirect calorimetry method. However, the disadvantage with this method is the artificial conditions of living in a closed environment. Its influence on M.R. of the individual is yet to be established (Jequier & Schutz, 1983).

The respirometers are used to collect expired air via tight fitting face mask or mouth piece. These may be uncomfortable but they have been shown to be tolerated by a wide range of subjects (Segal, 1987). When used properly they give results comparable to those obtained using either of the direct respiratory chamber methods. The ventilated hood system was first described by Benedict in 1930; the principle of this system is that a stream of air is forced into a transparent hood placed over the head of the subject and made air tight at the neck. The rate of metabolism can be determined by measuring the amount of air flowing through the hood and by measuring O_2 and CO_2 concentration in the inflow and outflow. This system is comfortable and it may be used to measure E.E. in patients without causing them much discomfort (Segal, 1987).

3.2. Non-calorimetric methods.

Non- calorimetry methods for estimating E.E. in man, include techniques based on physiological measurements, such as heart rate

and pulmonary ventilation; human observation and recording methods, such as time and motion studies and activity diaries; and kinematic recordings such as radar and mechanical activity meters. The errors inherent in these methods are too great to permit accurate measurement of E.E..

The double isotopically labelled water ($^2H_2$ ^{18}O), a simple and non-invasive method is currently being used to measure E.E. in free living individuals (Schoeller & Webb, 1984; Schoeller et al, 1986). This method allows subjects to perform their normal day-to-day activities, but it is expensive and requires access to a specialized mass spectrometer for analysis of the samples. This method is based on the observation made by Lifson in 1949 that the O_2 of the respiratory CO_2 mixes freely with the O_2 of body water. Therefore by measuring CO_2 production, E.E. can be estimated. However, this technique is based on a number of inherent assumptions: the body water volume is constant; rates of water flux and CO_2 production are constant; isotopes label only water and CO_2 in the body and they leave the body as water and CO_2; the isotopic enrichment in water and CO_2 leaving the body are the same as in the body water; water CO_2 do not enter the animal across the skin or lung surfaces. More research is required to establish the validity of these assumptions, particularly those related to the effects of fractionation and compartmentation of the isotopes (Jequier et al, 1987). The longer experiment period of about 5 to 14 days and relatively easy way of sample collection have encouraged wide application of the this technique.

JUSTIFICATION AND OBJECTIVES.

BMR prediction from body mass (B.M.) has great variability between individuals of the same sex and age range; other factors have been studied in order to improve its prediction but, the improvement has been so small that none has been included, probably because of practical reasons.

FFM has been proposed as a metabolic reference standard as differences between individuals of different sex, age, physical activity and race are lower once FFM is taken into account, but its use has not been found to significantly improve BMR prediction above B.M.. In lean and muscular individuals BMR may be further explained by other body composition (B.C.) variables related to muscularity, fatness and body frame.

Lean and muscular individuals, because of their differences in B.C. and physical activity may have a total energy expenditure (E.E.) during certain activities, mainly those that use skeletal muscle, different from individuals of more average body build.

The present study was performed on 157 lean and muscular, young (18-45 years), healthy and physically active women and men.

The aims of the present study were to:

1) investigate the part played by differences of B.C, in terms of B.M., height, fat mass, FFM, body potassium (i.e., muscularity), body girths and bone breadths,

2) evaluate B.M. and FFM in relation to their estimation of BMR, using three mathematical approaches: simple ratio standard, power function ratio standard and linear regression analysis,

3) study different B.C. variables to evaluate if either of them significantly increases BMR prediction above B.M. or FFM,

4) compare BMR measured and calculated by some of the most used predictive equations,

5) find the multiplicative factor of BMR to estimate treadmill walking metabolic rate (TWMR) with the data of this study and compare it with the FAO/WHO/UNU, 1985 estimation,

6) evaluate which variable among BMR, B.M. and FFM explains most of TWMR variation among subjects employing 3 mathematical models,

7) investigate the effect of TBK (i.e., muscularity) on TWMR, and

8) study the contribution to the prediction of TWMR of all other B.C. variables measured.

METHODS.

1. SUBJECTS.

1.1. Recruiting and criteria for acceptance of the subjects studied.

A total of 157 subjects, 79 females and 78 males, mostly students and staff of the University of Glasgow, voluntarily participated in this study.

Since the aim was to concentrate the study on fairly lean, muscular, healthy young adult and adult subjects (from 17 to 50 years), they were recruited among members of sports and professional dancing communities such as: the University of Glasgow Sports Center (Stevenson Hall), from the following Athletic Clubs: Scottish National Athletic Team, Westerlands, Maryhill Harriers, Glasgow University A.C., Clydebank A.C., Moir Ayr Seaforth and Victoria Park A.C.; from the Scottish Ballet Company and few subjects from the rowing, weight lifting and wrestling clubs.

The selection strategy was based on the regularity and intensity of sports and dancing practices and on the "lean" and "muscular" appearance of the subjects; there were some subjects that although not performing any specific sport were apparently lean and some others that practicing a sport on a regular basis were not apparently lean; both of these groups were also included.

Once identified, participants in this study were mainly recruited in either one of the following three ways:
1) personally approached and verbally explained the characteristics of the study; i.e., attending to Sports Centers and Competitions.
2) by sending a letter to all the above mentioned Athletic Clubs and through the distribution of leaflets to Sports Centers (Appendix 1).
3) by a formal talk in which the study was completely explained. i.e., the participants from the Scottish Ballet Company.

All subjects participated on a strictly voluntary basis; an informed consent letter was signed by them all.

They were all in apparent good health and reported no previous history of diabetes mellitus, thyroid disease or other metabolic disorders. None were receiving any drugs or medication.

The Scottish Ballet Company were all professional dancers devoting most of the day to practicing this activity.

Most of the subjects that participated in this study were runners (57% of the sample) and 7 of them were international competitors. Those attending the Stevenson Hall practiced different sports, mainly fitness sessions (30-40 minutes of mainly aerobic exercises of various types and intensities) and Shorinji kempo (japanese martial art) but also swimming, cycling, mountaineering, squash, basket ball, volley ball, weight lifting, soccer foot ball, rugby, skiing, wrestling and rowing.

Subjects were roughly classified according to the spare time intensity of physical activity. Intensity groups were:

- **light**, those subjects performing two hours or less per week of light activities such as walking, very slow jogging, badminton, etc,;
- **moderate**, those subjects performing 3 to 7 hours per week of sporting activities or exercise sessions, and
- **heavy**, formed by those subjects performing 8 or more hours (up to 40) per week.

Details of the subject's habitual activity were recorded.

2. DESCRIPTION OF THE METHODS EMPLOYED.

2.1. Measurement of Metabolic Rate (BMR and treadmill walking metabolic rate).

BMR and treadmill walking metabolic rate (TWMR) were determined by open circuit indirect calorimetry, using the Douglas bag technique, under carefully standardized conditions (in the morning, after an overnight fast, after a preliminary period of at least 30 min bed-rest at an equable temperature). BMR was calculated as the average of three 10-min measurements using Weir's equation (below described).

Calibration of the gas analysers. The **paramagnetic oxygen analyser** (servomex type 570A SYBRON, Servomex Ltd. Crowborough, Sussex, England) and the **infrared carbon dioxide analyser** (PK morgan Ltd., Chatham, Kent, England) were calibrated on each test prior to the start of the experiment. They were first set at zero by introducing O_2 free N_2 and then 'spanned' or calibrated using standard gas mixtures **"4.05% CO_2:16.30% O_2" or "6.06% CO_2:15.62% O_2" tested by Schollender** (British Oxygen Co. Ltd., Great Westhouse, Brentford, England). The span of the O_2 analyser was set at 20.93% using fresh dried atmospheric air. O_2-free N_2 was introduced again to reset the analysers at zero.

Apparatus. The set of instruments to measure M.R. consists of a **Douglas bag** which is a large gas impermeable plastic bag, of either 100 or 200 litres capacity (Cranlea & Co. Birminham, UK). This is connected via a **three way aluminum tap** to a length of **flexible corrugated plastic tubing** (length 122 cm, ID 2.86 cm), which is in turn attached to a **two way Rudolph low resistance valve No. 1400** (Kansas City MO. USA). A **rubber mouth piece** is fitted onto the Rudolph valve (figure 2.2.).

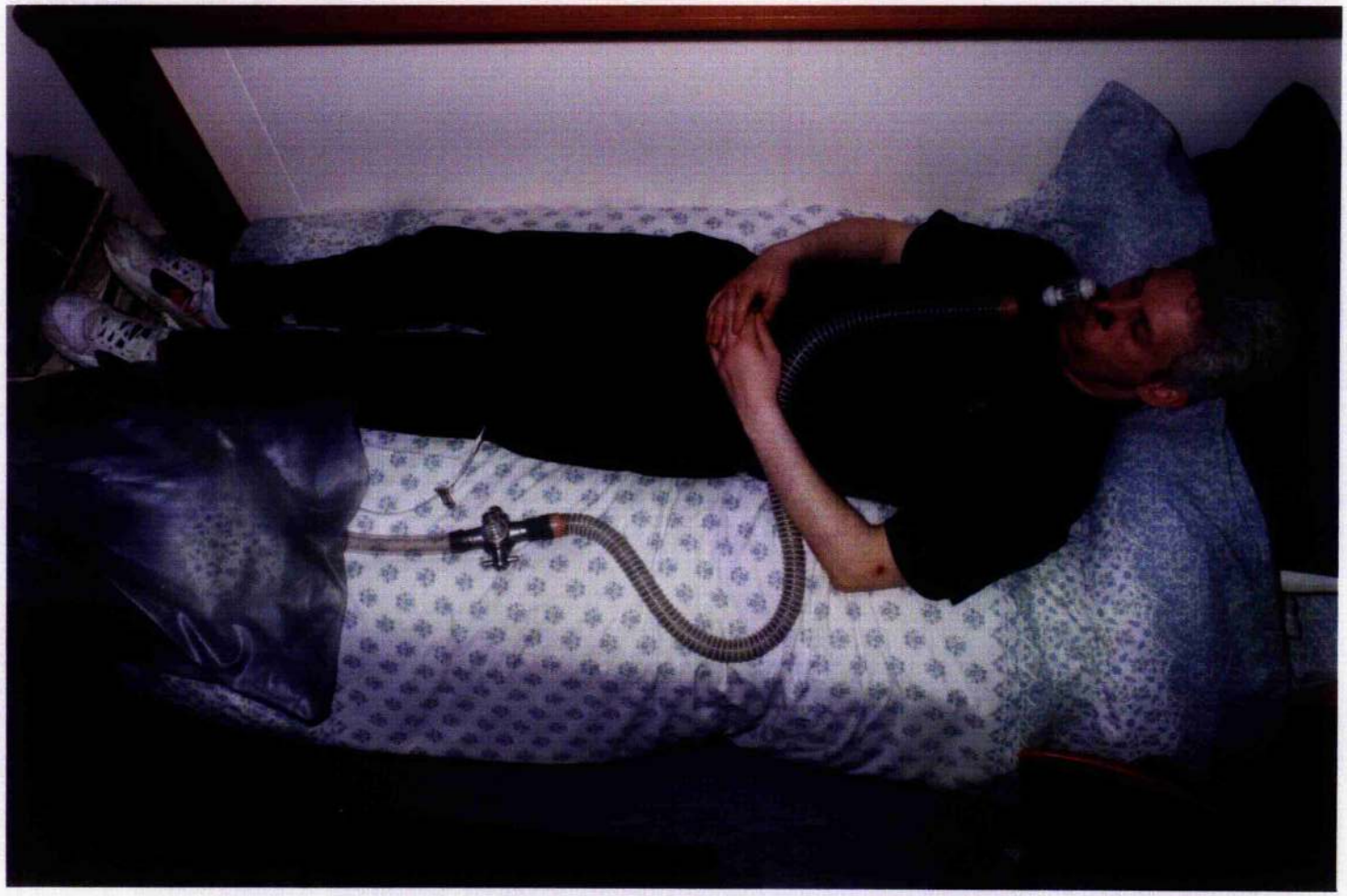

Figure 2.2. Measurement of basal metabolic rate. Set of instruments which form the apparatus and collection of expired gas by the Douglas bag system.

Collection of expired air. The subject's nose was fitted with a **nose clip**, so that he/she was able to breath only through the mouth piece connected to a two way Rudolph valve. This valve allows the subject to draw air from the atmosphere but all expired gas is directed through flexible plastic tubing toward the Douglas bag. Depending on the position of the three-way tap, interposed between the bag and one end of the plastic tubing, the expired air can either enter the collection gas or pass back to the atmosphere. For the first 3-5 minutes of a measurement the tap is in the latter position and expired air returns to the room. This allows the subject to be in respiratory equilibrium, to 'settle down' and become used to the breathing through the apparatus before the actual collection begins. After the initial run-in period the tap is opened and expired air collected into the Douglas bag for 10 minutes (figure 2.2.). The tap is then closed off, the bag disconnected from the breathing system and taken away for analysis of gases.

Analysis of expired air. A sample of expired gas was introduced into the analysers through a side tube attached to the Douglas bag. One minute was allowed for the reading of the analysers to stabilize (equivalent to 0.5 l of gas passing from the bag) and the CO_2 and O_2 contents recorded. The side tube was closed off and the volume of the expired air was measured using a gas meter (Parkinson-Cowan Ltd., London, England), taking into account the 0.5l already used for analysis. The temperature of the expired air was recorded using a thermistor attached to the gas meter. The volume of expired air (pulmonary ventilation) was corrected for the amount of water vapour (saturation) at standard temperature and pressure, dry (STPD), using an appropriate 'atmospheric correction factor: F' from a standard nomogram on the basis of barometric pressure and temperature (BTPS) (Consolazio et al, 1963).

2.2. Calculation of Metabolic Rate.

Metabolic rate (M.R.) was calculated according to the following equation:

M.R. [kcal/min] = O_2 consumption [L/min] * Calorific equivalent O_2 [kcal/L]

and

Oxygen consumption = 'true' oxygen * ventilation rate

Ventilation rate (V.R.) is equal to the total volume of air expired per minute, and is usually expressed as litres (L) of dry air at STPD. It is obtained by multiplying the metered volume (Vol.) of

expired air by F and dividing this by the duration of the sample collection:

$$V.R.[L/\text{min}][STPD] = \frac{Vol.[L] \times F}{Sample\text{-}duration[\text{min}]}$$

If the volume of the inspired air is equal to the volume of expired air, the O_2 consumption can be obtained simply from the difference between the volume of O_2 inspired and that expired:

O_2 consumption = (Vol. O_2 inspired - Vol. O_2 expired)

$$= \frac{20.93}{100} \times V_i - \frac{\%O_{2e}}{100} \times V_e$$

equation 1

where:

V_i = volume of air inspired;

V_e = volume of air expired;

20.93 = $\%O_2$ in inspired air;

$\%O_{2e}$ = $\%O_2$ in expired air.

However, when V_i and V_e are not equal, as is the case when the R.Q. is less than 1, an adjustment is required to derive the 'true' value for the O_2 difference. This computation is based on the fact that the volume of N_2 breathed in (Ni) will always equal the volume of N_2 breathed out (N_e):

$$V_i \times \frac{N_i}{100} = V_e \times \frac{\%N_e}{100}$$

where: N_i = 79.04, and

$$V_i = V_e \times \frac{\%N_e}{79.04}$$

Taking equation 1 and substituting:

$$O_2 consumption = \frac{20.93}{100} \times V_e \times \frac{\%N_e}{79.04} - \frac{\%O_{2e}}{100} \times V_e$$

$$= V_e \times \frac{20.93 \times \%N_e}{100 \times 79.04} - \frac{\%O_{2e}}{100}$$

'true' oxygen

Thus the 'true' oxygen value can be derived, and when multiplied by V.R. gives a measure of O_2 consumption. In this study 'true' oxygen, was not calculated as above, but obtained using a nomogram (Consolazio et al, 1963).

Finally, M.R. was calculated according to Weir's equation, 1949. Previously, the estimation of M.R. by indirect calorimetry involved measuring urinary N_2 excretion, in addition to gaseous exchange, in order to determine the proportions of the different nutrients oxidized in the body. The calculations involved were often so cumbersome that the effect of protein was commonly ignored. In 1949, Weir developed an equation which took into account the effect of protein metabolism, without the necessity of having to measure N_2 excretion. The equation is based on the assumption that a fixed percentage (mean 12.5; 11-14%) of the total calories expended by the body arise from protein metabolism and of an R.Q. equal to 1. Weir then calculated that the amount of heat released for every litre of O_2 used, the calorific equivalent of O_2, would be 5 kcal/L. Thus,

$$\mathbf{M.\ R.\ =\ 20.93\ -\ \%O_2\ *\ V_e\ *\ 5}$$

$$M.R. = \frac{20.93 - \%O_{2e}}{20} \times V_e$$

If however, the R.Q. is less than 1 (and consequently V_e is less than V_i) the volume of O_2 inspired, and therefore the M.R. calculated according to this equation, will be under-estimated. However, as R.Q. falls the calorific equivalent of O_2 also falls tending to over-estimate M.R.. Under normal circumstances these two errors cancel out and the Weir's equation gives an accurate assessment of M.R..

2.3. Determination of Basal Metabolic Rate.

BMR is defined, in this thesis, as the rate of energy produced under the standardized resting conditions outlined by Benedict, 1938 which are: lying awake in a supine position, at complete physical rest; postabsorptive, at least 12 hours after the last meal; in a thermoneutral state; emotionally undisturbed; without disease or fever. In all measurements of BMR every attempt was made to meet each of the above criteria.

Some confusion about the exact way in which BMR should be measured has been put forward by Schutz, 1984, among others. The impression seems to have arisen that for a measurement to be properly defined as basal it should be made just after wakening, prior to the subject getting out of bed, so the subject has been admitted to a metabolic unit or to a suitable place where the measurement of BMR is to be carried out the following morning. Whatever the rationale of this view, these are not certainly the ones under which almost all the fundamental work on BMR has been carried out (Benedict, 1915; Du Bois, 1927; Boothy et al, 1936; Benedict, 1938). In all these cases subjects were not required to stay overnight before a BMR measurement. The same as in this study, subjects arrived at the laboratory early in the morning after spending the night in their own homes.

Instructions for subjects. Subjects were instructed not to eat or drink anything from 9.00 pm on the day preceding their measurement and to continue the fast on the morning of the test.

Some doubts have arisen as to the length of time that the thermic effect of food (TEF) lasts; one of the obvious answers is that the duration of the TEF will depend on the composition and size of the meal consumed; caffeine and alcohol have also shown to have effects on BMR.

Subjects were then warned on these aspects and were asked to have their accustomed dinner preferably before 7:00 pm, not to overeat and abstain from drinking caffeine and alcohol beverages.

Regarding exercise, subjects were also instructed to refrain from any strenuous activities on the previous day of their measurement. As many of the subjects of this study practiced some kind of physical activity almost on a daily basis, and even some were professionals, this restriction was not always well accepted and even some subjects would not participate at all because of this.

Subjects were also asked to try to rest as much as they could and have a good sleep; on the day of the experiment, they were asked to take it easy to get to the lab., even if they had to walk or come by bus, they were asked not to hurry, not to jog nor to cycle.

Procedure for the measurement of BMR. Subjects reported at the laboratory between 8 and 8:30 am; once there, they were asked to empty their bladder, wear a gown, weighed and asked to lay in a supine position, without moving, in a coach to rest for 30 minutes before the measurement procedure began, to allow M.R. to return to a basal level. The room was maintained at about 20°C ($\pm$ 2), so that the was comfortable.

The entire measurement was explained to the subjects and they were shown and familiarized themselves with the equipment. This helped to ensure that they were as relaxed and confident as possible.

Once all the above pre-measurement procedure was over, the subject was fitted with a nose clip and a mouth piece. A 5 minute run-in period followed, allowing the subject to become accustomed to breathing through the apparatus. Then, three 10 minute collections of expired air were made, allowing the subject to rest for a few minutes, i.e., take out the nose clip and mouth piece, between the second and the third measurement, which was preceded by a 3 minute run-in period. At the end of each collection heart rate (H.R.) was measured using the radial pulse. Air content of the bags were analysed and M.R. calculated as described above. The average M.R. of these three measurements was taken to represent the BMR of the subject.

2.4. Comparison between BMR measured and estimated by widely used predictive equations.

BMR was calculated with the following equations:

- **WHO**, 1985;

Females	(18-30 yrs.):	**14.7 * B.M. + 496**
	(30-60 yrs.):	**8.7 * B.M. + 829**
Males	(18-30 yrs.):	**15.3 * B.M. + 679**
	(30-60 yrs.):	**11.6 * B.M. + 879**

- **Kleiber**, 1947

Females: $65.8 * BM^{.75}$, [1+[0.004(30-age)] + 0.018 * (S-42.1)

Males: $71.2 * BM^{.75}$, [1+[0.004(30-age)] + 0.01 * (S-43.4)]

where:

BM = body mass [kg]; age [years]; S = height [cm]/ $BM^{.33}$ (specific stature in $cm^{1/3}$; assuming each additional cm per $kg^{1/3}$ in specific stature produces an average increase of 1% of the M.R. of men and 1.8 % of the M.R. of women).

- **Harris & Benedict**, 1919

Females: **655.096 + 9.56*BM[kg] + 1.85*Ht[cm] - 4.676*age [yrs.]**

Males: **64.473 + 13.752*BM[kg] + 5.003*Ht[cm] - 6.755*age[yrs]**

- **Cunningham**, 1980

501 + 21.6 * FFM [kg]

- **Dubois & Dubois**, 1916 derived a well accepted equation to obtain body surface area (BSA). The energy expressed in relation to BSA

[kcal/day/m^2] is a customary clinical practice. This relationship is based on the assumption that heat loss and therefore BMR are proportional to BSA. The Mayo Foundation has derived simple, easy to use tables: Normal Standards Calories per m^2 per hour to estimate BMR (Pike & Brown, 1975).

$$\mathbf{BMR = BSA * F}$$

where:

BSA = $BM^{.425}$ [kg] * $Ht^{.725}$ [cm] * 71.84;

F = factor depending on age, for example:

AGE	FEMALES	AGE	MALES
17	37.82	17	44.80
18-19	36.74	18	43.25
20-24	36.18	20-21	41.43
25-44	35.70	22-23	40.82
45-49	34.94	24-27	40.24
50-54	33.96	30-34	39.34
55-59	33.18	35-39	38.68
		40-44	38.0
		45-49	37.37

2.5. Determination of the Energy Expenditure of walking on the treadmill.

Procedure. Once BMR had been measured, subjects were measured their M.R. when walking on a **motor driven treadmill** (Quinton Instruments Company, Seattle, Washington) under standardized conditions: in a fasted state, for a period of 8 min, at a treadmill speed of 4.8 km/h and 0% slope.

Subjects were allowed 3 minutes or so at the beginning of walking in order to get used to walking on the treadmill. After this period, once the said that she/he was feeling confident, a run-in period of 5 min was allowed to achieve a steady state. Meanwhile, they were asked to fit a nose clip and the mouth piece attached to the gas collection apparatus so that they could breath normally through it and get ready for gas collection. Finally, two 8 minute samples of expired air were collected in 200 litres Douglas bags (figure 2.3.). The expired gas was analysed for O_2 and CO_2 content as previously described. M.R. was expressed as the mean of the two readings.

Heart rate was measured during the gas sample collections using a **heart rate monitoring apparatus** (Hewlett Packard 1/10 40493 E), three monitoring electrodes (Ag/AgCl) were appropriately fitted in the chest.

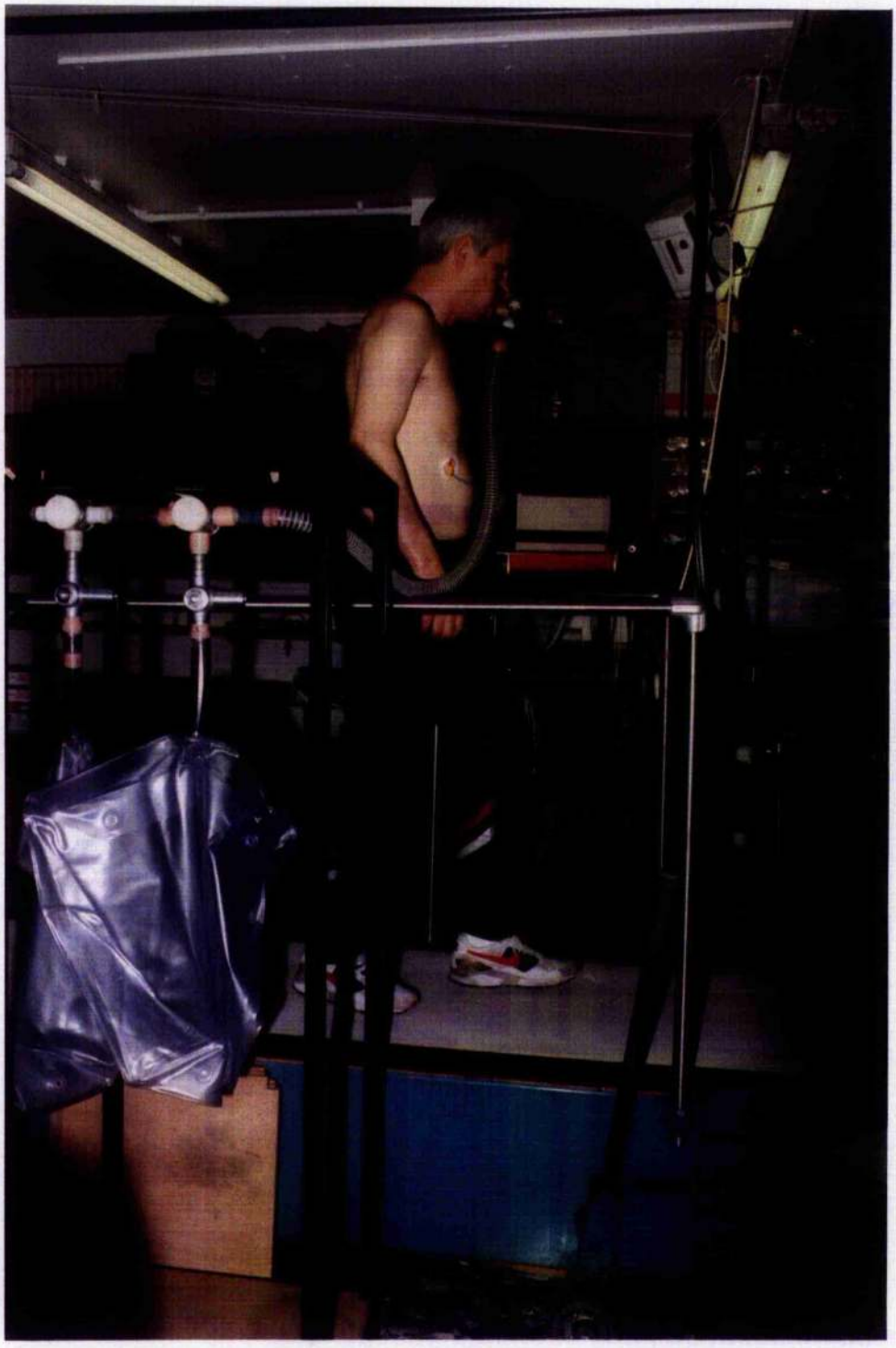

Figure 2.3. Measurement of metabolic rate of walking on a motor driven treadmill. Apparatus and collection of expired gas.

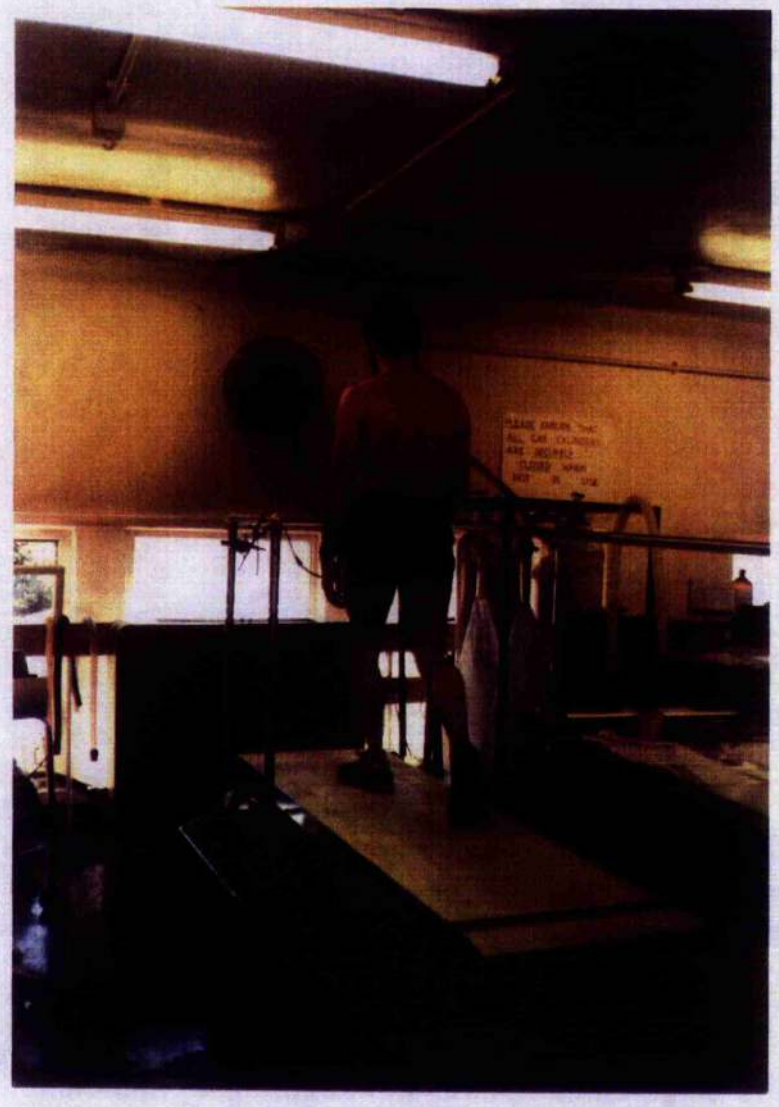

Figure 2.3. (continued). Subjects are shown while walking on the motor driven treadmill at 4.8 km/hr, wearing the nose clip, connected to the air collection apparatus and wearing the electrodes on the chest.

2.6. Measurement of Body Composition.

Measurements of body mass (B.M.), height, body girths, bone diameters, skinfold (SkF) thickness and body density (B.D.) by underwater weighing were assessed as described in the methods section of the B.C. chapter. Body fat content was estimated from the sum of four SkF thicknesses (Σ4SkF: biceps, triceps, supra-iliac and subscapular) according to the equations of Durnin and Womersley, 1974 and from B.D.

2.7. Statistical Methods.

Linear regression analyses were performed to derive equations to predict BMR from B.M. and from FFM (estimated by three methods); 98% confidence intervals (C.I.) for the mean difference between each pair of BMR/FFM were assessed and the limits of agreement (mean difference ± 2 S.D.) were calculated to set the limits where most of the differences are expected to lie.

Correlation analyses were performed for each sex, between BMR per kg of BM on BM and between BMR per kg of FFM on FFM.

Simple ratio standards were performed using the no-constant option and the curvilinear power function ratio standards assuming a log-linear power model to estimate the parameters. The logarithmic transformation of both variables ('x and y') were assessed to find the power function and evaluated by analysis of variance to assess statistical significance between sexes. Then linear regressions were performed using the power function found.

The linear relationships between BMR and the various anthropometric and other B.C. variables were assessed using univariate regression analyses.

Stepwise multiple regression analyses were performed to find out which of the B.C. variables best explained differences in BMR.

In order to find the variables that best predicted treadmill walking metabolic rate (TWMR) the following was performed:

- The BMR multiplicative factor was looked for.
- Linear regression analysis to predict TWMR per kg of B.M. and per kg of FFM.
- An ANOVA was performed of TWMR on BMR, B.M. and FFM to find out which of these three variables best predicted TWMR and whether either of them adds something else to the prediction.
- A stepwise multiple regression analysis was performed to find out which of the B.C. variables best explained TWMR.

RESULTS AND DISCUSSION.

1. DESCRIPTION AND DISTRIBUTION OF THE DATA.

The anthropometric data and the results of the basal metabolic rate (BMR) and the metabolic rate of walking on the treadmill (TWMR) of the subjects that participated in this study are presented in table 2.1.

There were 8 individuals, 3 women and 5 men older than 40 years. The range of values of all variables of the younger subjects include the values of the older ones and the mean values with and without their data was not significantly different; therefore, these older subjects were included with the whole group.

BMR can vary enormously between individuals; an almost two fold range within sexes was found in this study between the largest and smallest values and almost a three fold range if both sexes were studied together. About 600 kcal/day separated the 5% of the subjects at the top end of the range from the 5% at the bottom. This fact is not surprising; other studies have reported that in healthy adults BMR can vary over a three fold range between individuals of different races, sexes, B.M. and age (Harris & Benedict, 1919; Ravussin et al, 1986).

It may also be seen in tables 2.2.A & B that variation is smaller when sex is taken into account and when BMR is expressed per kg of B.M. or on a FFM basis but still, almost 10 units separated subjects from the 5% at the top and at the bottom edges. These facts shall be discussed below.

VARIABLE	FEMALES (n = 79)	MALES (n = 78)
AGE [years]	26.6 ± 7.1 (16-63)	26.2 ± 8.1 (17-53)
BODY MASS [kg]	55.4 ± 6.4 (40.2-68.2)	68.8 ± 8.2 (53.3-92.2)
HEIGHT [cm]	165.2 ± 6.1 (150.2-178.9)	179.7 ± 7.3 (162.5-195.8)
B M I [kg/m^2]	20.2 ± 1.69 (16.1-24.2)	21.3 ± 2.38 (17-29.7)
TRICEPS SkF	11.9 ± 3.22 (5-19)	6.9 ± 2.14 (4-14)
BICEPS SkF	5.2 ± 1.96 (2-11)	3.2 ± 0.77 (2-5)
SUBSCAPULAR SkF	9.8 ± 2.67 (4-17)	8.9 ± 2.13 (6-18)
SUPRAILIAC SkF	8.6 ± 3.32 (4-17)	8.9 ± 3.89 (2-26)
ARM CIRCUMFERENCE	24.9 ± 1.84 (20.7-28.8)	28.5 ± 2.96 (23.5-40.9)
WAIST CIRCUMFERENCE	66.2 ± 5.03 (55.4-85)	75.3 ± 4.73 (65.5-88)
BUTTOCKS CIRCUMFERENCE	91.8 ± 4.4 (79.3-101.6)	93.6 ± 4.23 (85-103.8)
THIGH CIRCUMFERENCE	53.2 ± 3.63 (45-60.5)	53.9 ± 4.16 (35-65.2)
CALF CIRCUMFERENCE	34.9 ± 2.24 (29.2-41)	36.9 ± 2.38 (32-42.8)
BIACROMIAL DIAMETER	36.8 ± 1.89 (28.3-40.1)	40.7 ± 2.18 (36.5-46)
BIILIAC DIAMETER	28.1 ± 2.09 (22-37)	28.4 ± 1.90 (24.4-33.7)
BIESTILEON DIAMETER	5.1 ± 0.28 (4.4-6.1)	5.8 ± 0.46 (4.9-8)
FEMORAL DIAMETER	8.8 ± 0.48 (7.7-9.8)	9.6 ± 0.69 (8.5-12.0)
BODY DENSITY [g/cm^3]	1.0541 ± 0.0102 (1.035-1.088)	1.0746 ± 0.0089 (1.049-1.089)
BODY POTASSIUM [mmol]	(n=28) 2935 ± 322.6 (2113-3615)	(n=38) 4206 ± 545.5 (3244-5387)
B M R [kcal/min]	0.929 ± 0.1156 (0.61-1.16)	1.259 ± 0.1496 (0.96-1.74)
TWMR [kcal/min]	2.945 ± 0.3748 (2.055-3.68)	4.513 ± 0.5364 (3.45-5.95)

Table 2.1. General data of the subjects studied. Mean ± S.D. (Min-max)

VARIABLE	MEAN ± S.D. (MIN-MAX)	p 5%	p 25%	p 50%	p 75%	p95%
B M R [kcal/day]	1338.3± 166.4 (878.4-1670.4)	1022	1238	1339	1483	1598
BMR / BM [kcal/day/kg]	24.2 ± 2.34 (19.3-29.7)	20.6	22.5	24.3	25.9	28.7
BMR / FFM [kcal/day/kg]	30.2 ± 2.73 (24.2-37.7)	25.6	28.2	30.5	32.0	35.0

Table 2.2.A. Distribution of the data. Females (n = 78)

B M R [kcal/day]	1813.7 ± 215.47 (1382.4-2505.6)	1512	1670	1771	1930	2160
BMR / BM [kcal/day/kg]	26.5 ± 2.71 (21.1-33.7)	22.2	24.7	26.2	28.6	31.1
BMR / FFM [kcal/day/kg]	29.7 ± 3.02 (24.2-37.4)	25.0	27.8	29.0	32.1	35.8

Table 2.2.B. Distribution of the data. Males (n = 78).
BM = body mass; FFM estimated by densitometry.

2. RESULTS ON BMR.

2.1. BMR related to Body Mass.

As expected, B.M. was found to be significantly related to BMR.

Linear Regression. The regression analysis of BMR [kcal/day] on B.M. [kg] showed to be different for each sex (fig. 2.4); the following equations were obtained:

FEMALES (n = 79) BMR = 338.3 (± 118.5) + 18.05 * B.M. (± 2.1);
r^2 = 0.48; r = 0.70; RSD = 120.36

MALES (n = 78) BMR = 685.5 (± 163.2) + 16.4 * B.M. (± 16.4);
r^2 = 0.39; r = 0.62; RSD = 169.47

The ANOVA showed the coefficients of the regression equations to be significantly different between sexes but the slopes or constant multipliers of B.M., were not. Then, the regression was performed allowing different coefficients but a constant slope.

FEMALES (n=79) BMR = 394.7 (± 90.14) + 17.03 * BM (± 1.6);
RSD = 119.8

MALES (n=78) BMR = 642.1 (± 111.3) + 17.03 * BM (± 1.6);
RSD = 168.4

For both sexes (n=157); RSD = 146.8; r^2 = 0.774

Comparing these equations with the FAO/WHO/UNU, 1985 equations, i.e., 18-30 years for females: 496 + 14.7 * B.M. and for males 679 + 15.3 * B.M., it can be seen that both look similar. The computation of the FAO/WHO/UNU equations for the subjects of this study showed, for females a non significant mean difference but a significant one for males. For both sexes important individual differences were found (see table 2.10).

Simple Ratio Standard (BMR/BM). The ANOVA showed the simple ratio standards to differ between sexes and thus were obtained for each sex by regression with the no-constant option; the obtained ratios were as follows:

FEMALES (n=79) BMR = 24.08 * B.M. (± 0.25); RSD = 125.8
MALES (n=78) BMR = 26.23 * B.M. (± 0.31); RSD = 186.9

The errors of prediction in these cases were larger than including a constant value; the use of these ratio standards shall be discussed later.

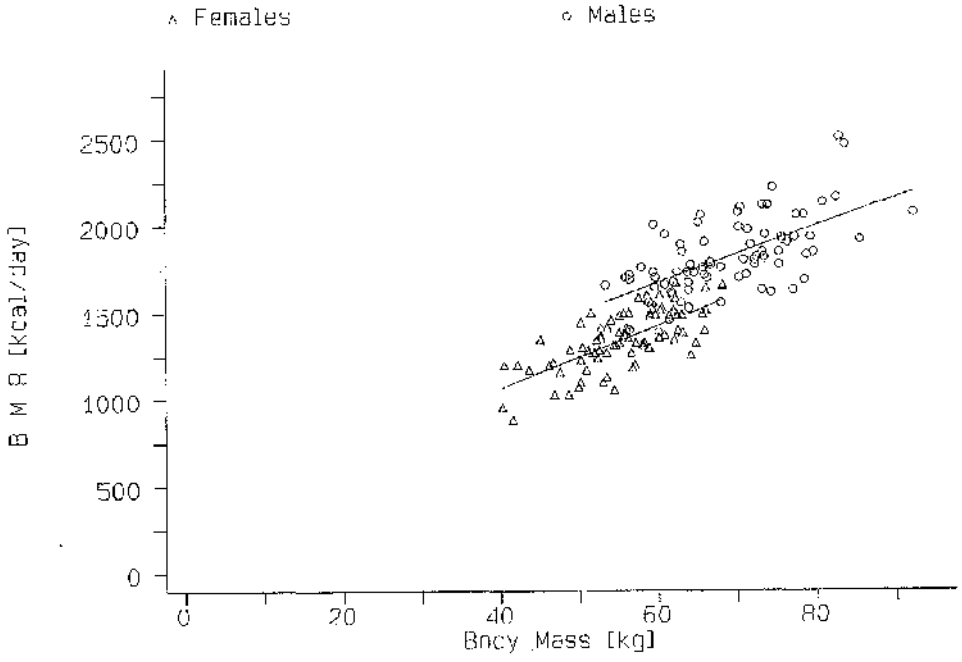

Figure 2.4. Regression of BMR on body mass for females (n=79) & for males (n=78).

B.M., in this group of subjects, explained 48% of BMR variability in females and 39% in males.

Power Function Ratio Standard. The relationship between BMR and B.M. was not linear as the regression line did not pass through the origin for either sex; the intercept parameters for both sexes were found to be significantly greater than zero. Then, the use of the power function ratio standard (P.F.R.St.) model was analysed.

The power function parameter estimated by ANOVA was found to be not statistically significant different between sexes (i.e., 0.685; $p = 0.24$). Therefore the same value was used for both sexes.

When BMR was regressed on $BM^{.69}$ the constant multipliers were found to be different between sexes. Then, the data was analysed together allowing a different constant for each sex but a common power function parameter (figure 2.5). The resulting power function equations were: BMR [kcal/day] =

FEMALES (n=79): $85.56(\pm 0.86) * B.M.^{.69}$; RSD = 119.76
MALES (n=78): $99.86(\pm 1.05) * B.M.^{.69}$; RSD = 169.07

It may be seen that the r^2 was not included neither for the simple ratio standard nor for the P.F.R.St. models. When a regression analysis is performed the ANOVA table and its associated statistics are adjusted for the explanatory power of the constant. The regression in effect had a constant; when the no-constant option is selected, no such adjustment is made and all the explanatory power is left to the chosen variable, in this case B.M.; the result is that the ANOVA showed a $r^2 > 0.99$, for both sexes, which seems too high since the error of prediction using either the linear regression or the P.F.R.St. was very similar and the r^2 for the linear regression was of only 0.48 for females and 0.39 for males.

The best fit may be considered to be the model that gave the least error associated with the prediction, i.e., RSD [kcal/day].

	Females	Males	Both
Simple Ratio	125.8	189.9	159.1
Linear			146.5
Regression	119.8	168.4	
Power Function	119.8	169.1	146.4

The simple ratio standard clearly gave the greatest prediction error; furthermore, the use of simple ratio standard would not be correct, because the condition of equality between the relation: coefficient of variation (CV) of the x variable on the CV of the y variable $(CV)_x / (CV_y)$ and the coefficient of correlation (r) was not achieved by either sex, i.e.,

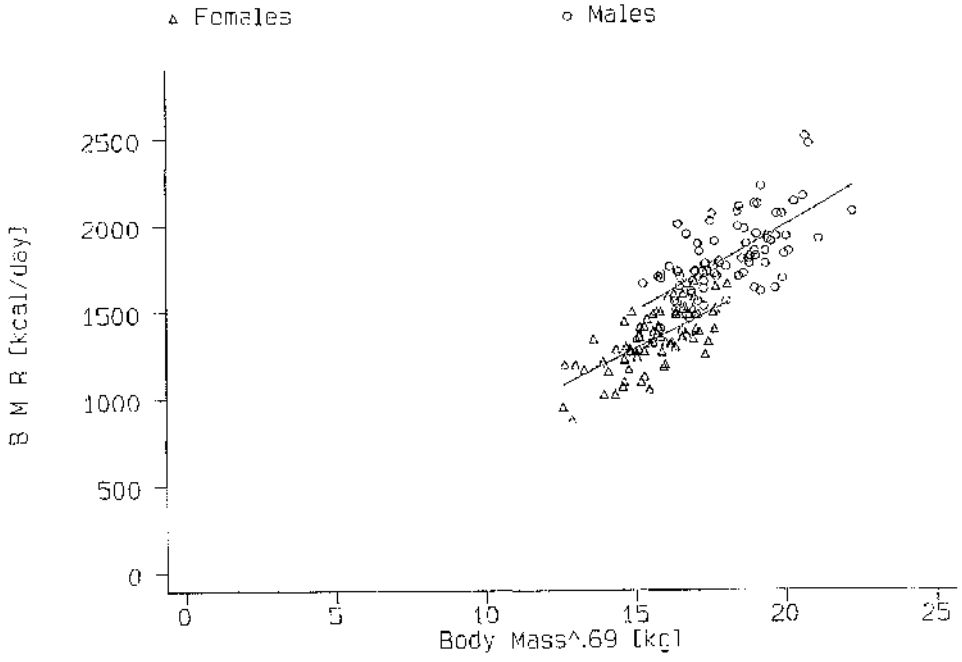

Figure 2.5. Power function ratio standard of BMR on body mass for females and for males.

Females: CV_x / CV_y: 11.6/12.4 = 0.93 and r = 0.70, and
Males: CV_x / CV_y: 11.9/11.9 = 1.0 and r = 0.62.

The prediction error was practically the same using either the linear regression or the power function scaling model; but, theoretically, the relationship between BMR and B.M. is more likely to be a power function rather than a linear model because the relationship between these two variables was not linear as it could be seen that the regression line did nor pass through the origin. Support for the use of power function models comes since the work of Kleiber, 1947, 1950 and Schmidt-Nielsen, 1984, that have proposed its use to explain the relationship between a physiological variable and a body size variable for subjects and animals of different sizes. Probably, when comparing subjects with widely different body composition the use of the P.F.R.St. presents more advantages but, for these, fairly homogeneous groups of females and males the level of prediction of either model is the same.

Variation of BMR with Body Mass. For a given value of B.M. $\pm$ 2.5 kg (ie, B.M. values each 5 kg) there were found BMR values with large variations (table 2.3.).

Within these groups by B.M., there were not statistically significant differences in the BMR but in the 65-70 kg group of males (n=10) which showed a $p<0.02$ & $r=-0.72$; at a B.M. of 65 kg there were two subjects with very high BMR values and the other 8 subjects had lower and more similar BMR's to the rest; then, it appeared as a negative relationship between B.M. and BMR. If the B.M. range was instead of 10 kg, i.e., from 60-70 kg or 65-75 kg, there was a positive relationship & non statistical significant difference in the BMR within the groups. The B.M. range of these sub-groups was of 5 kg but any other range could have been chosen; the cutting point was arbitrarily selected and while a given group may present no statistical difference in their BMR, any movement in the number of cases may change the statistical significance.

The mean coefficient of variation (C.V.) of the BMR in these subjects was of 12.4% for females and 11.9% for males and within B.M. sub-groups, the Cs.V. ranged from 7-14% for females and from about 6-13% for males. No particular trend was found.

Groups by Body Mass [kg]	Females n =	Females BMR BMR/B.M. X ± SD (range)	Females C.V. [%]	Males n =	Males BMR BMR/B.M. X ± S.D. (range)	Males C.V. [%]
40-44.9	5	1077 ± 151.2 (878-1195)	14.0	-	-	-
		25.9 ± 3.45 (21.1-29.5)	13.3			
45-49.9	8	1161 ± 118.0 (1022-1339)	10.2	-	-	-
		24.5 ± 3.0 (21.0-29.7)	12.2			
50-54.9	23	1285 ± 120.6 (1051-1498)	9.4	-	-	-
		24.6 ± 2.4 (19.3-29.1)	9.7			
55-59.9	21	1389 ± 117.3 (1180-1598)	8.4	12	1661 ± 147.0 (1397-2002)	12.7
		24.3 ± 2.06 (20.8-27.5)	8.5		28.6 ± 2.58 (24.8-33.7)	9.0
60-64.9	17	1448 ± 113.2 (1253-1670)	7.8	17	1670 ± 147.0 (1382-1944)	8.8
		23.4 ± 2.04 (19.5-26.8)	8.7		26.7 ± 2.29 (23.0-31.9)	8.6
65-69.9	5	1541 ± 108.2 (1397-1656)	7.0	10	1801 ± 151.4 (1555-2059)	8.4
		23.2 ± 1.42 (21.2-24.8)	6.1		27.2 ± 2.60 (22.9-31.5)	9.6
70-74.9	-	-	-	19	1890 ± 177.3 (1613-2218)	6.2
					26.2 ± 2.49 (21.7-29.9)	9.5
75-79.9	-	-	-	13	1871 ± 126.3 (1627-2059)	6.8
					24.2 ± 1.67 (21.1-26.6)	6.9
80-84.9	-	-	-	4	2315 ± 196.5 (2131-2506)	8.5
					28.1 ± 2.08 (26.2-30.2)	7.4
ALL 40.2-68.2	79	1338 ± 166.4 (878 -1670)	12.4	-	-	-
		24.2 ± 2.34 (19.3-29.7)	9.7			
ALL 53.3-92.2	-	-	-	78	1814 ± 215.5 (1382-2506)	11.9
					26.5 ± 2.71 (21.1-33.7)	10.2

Table 2.3. Variation of BMR [kcal] with body mass [kg].

The one-way analysis of variance showed the comparison of BMR between groups by B.M., to be significantly different ($p<0.0001$) for both sexes. For females, statistical significant differences were found between most sub-groups (9 out of 15 comparisons, 60%) but any two adjacent groups. For example, the group 45-49.9 did not present significant difference in their BMR to the group 50-54.9, but it presented significant difference to the group 55-59.9. For males, most differences between sub-groups were not significant (26 out of 35 comparisons, 74%). Two sub-groups, the 65-70 and 70-75, were particularly different to the others. Two men with 'abnormally high' BMR values (>2300 kcal), were identified in the 65-69.9 group but their exclusion did not practically change the results.

It can also be seen in table 2.3. that when values were standardized for B.M. the Cs.V. were practically the same.

Relationship between BMR expressed per kg of body mass (BMR/BM [kcal/kg]) with body mass. It has been reported that the correlation between the simple ratio standard BMR/BM [kcal/kg/day] with B.M. gives a significant negative correlation (Lawrence et al, 1988); in the present study the same was found, i.e., $r=-0.32$; $p=0.0046$ for females and $r=-0.44$; $p=0.0001$ for males.

However, this fact is to be expected since it has been explained by Katch & Katch, 1974 & Winter, 1991, among others, that the simple ratio standards does not produce a dimensionless physiological variable; on the contrary they "over-scale" by converting a positive correlation to a negative one. What has been proposed instead by these authors is the use of regression analysis or of a ratio power function model. Lawrence et al, 1988 proposed the use of a power function model to better explain differences in BMR between light, average and heavy subjects and found for Gambian women the power function to be 0.5, which is different to the one found in this study, i.e., ≈0.7, for fairly lean women and men. Once BMR is scaled using the ratio power function model, i.e. $BM^{.68}$, the negative correlation became $r = 0.08$, n.s., for females and $r = -0.09$, n.s., for males.

2.2. BMR related to FFM.

The results of FFM by three methods were available.

FFM estimated by densitometry by underwater weighing (D-UWW) was estimated under the assumption of constant density values of the fat mass and the FFM; total body potassium (TBK) was estimated considering 60 and 68 [mmol/kg] as the amount of potassium of the FFM (K/FFM) for females and males respectively and FFM by skinfolds (SkF)

was estimated using the equations of Durnin and Womersley, 1974, (for the detailed assumptions of each method, see B.C. chapter, literature review, sections 5-8).

Table 2.4. shows the results of the BMR expressed per kg of FFM obtained by the three methods employed.

In order to have the same subjects for the comparison, only those subjects that had their TBK measured were included (n=65) because it was thought convenient to have the same subjects for the comparison.

As it can be seen, once the values were expressed on a FFM basis the difference between sexes became almost nil and not significant except when FFM was estimated by TBK. When 64 mmol of K per kg of FFM was used instead of 60 for females (the fact here is that calculation of FFM using 60 mmol per kg of FFM was not a correct way for calculating FFM in this group of lean-muscular females, see B.C. chapter, discussion, section 1.3.) the difference between sexes became not significant, i.e., the mean became 29.7 $\pm$ 2.30 (25.6-36.0) p = 0.96.

It has been observed in several studies (Cunninham, 1980; Bernstein, et al, 1983; Ravussin et al, 1986; Lawrence et al, 1988) that once the size of FFM is taken into account differences in BMR between groups of individuals, of differing age, sex and race are largely eliminated.

The results of table 2.5. show that, within each sex, differences may seem small but they were significantly different in all cases for females and for males all but the comparison between D-UWW and TBK. These differences obey to differences in the prediction of the FFM (this topic is widely discussed in the B.C. chapter; results and discussion). The most apparent difference was found with FFM estimated by TBK in females; when 64 mmol per kg of FFM was used instead, the difference between D-UWW and TBK became 0.18 $\pm$ 1.76 (range: -3.8 to 3.5), the 98% CI = -0.66 to 1.02, a not significant difference.

METHOD	FEMALES (n=27) [kcal/kg/day]	MALES (n=38) [kcal/kg/day]	"t-test" p =
BMR / BM	23.7 ± 1.88 (19.5-26.9)	26.9 ± 2.84 (21.5-33.7)	0.001
BMR / FFM (UWW)	29.8 ± 2.13 (25.6-33.3)	30.0 ± 3.11 (24.3-37.4)	0.88
BMR / FFM (TBK)	27.8 ± 2.28 (24.0-33.8)	29.8 ± 3.16 (24.2-38.4)	0.009
BMR / FFM (SkF)	30.7 ± 2.34 (26.0-35.4)	30.7 ± 2.89 (24.3-37.5)	0.93

Table 2.4. BMR variation in relation to the FFM estimated by different methods.
Values represent mean ± 1 S.D. (range).

	FEMALES (n=27)		MALES (n=38)	
DIFF	X DIFF. ± S.D. (Min- Max)	98 % CI & (Limits of Agreement)	X DIFF. ± S.D. (Min- Max)	98 % CI & (Limits of Agreement)
D- K	2.03± 1.69 (-1.6 - 5.2)	1.23 - 2.84 (-1.3 - 5.4)	0.20 ± 1.47 (-3.5 - 2.9)	-0.38 - 0.77 (-2.7 - 3.1)
D- S	-0.93± 1.33 (-3.2 - 2.2)	-0.6 - -0.3 (-3.6 - 1.7)	-0.68 ± 1.11 (-3.3 - 1.2)	-1.12 - -0.25 (-2.9 - 2.2)
K- S	-2.96± 1.67 (-6.8 - 1.2)	-3.8 - -2.2 (-8.3 - 0.4)	-0.88 ± 1.58 (-4.9 - 2.9)	-0.26 - 0.53 (-4.0 - 2.3)

Table 2.5. Mean difference (±S.D.), 98% Confidence Interval and limits of agreement for the mean difference [kcal/kg/day] of BMR expressed per kg of FFM estimated by different methods.

X DIFF. = Mean Difference between methods: D = Densitometry; K = Potassium; S = Skinfolds. 98% CI = Confidence interval for the mean difference. Limits of Agreement = mean difference ± 2 S.D.

Relationship between BMR per kg of FFM (BMR/FFM) with body mass and FFM estimated by different methods. Lawrence et al, 1988 have found that BMR/FFM has a tendency to fall from light to heavy individuals. They concede however, that this observation may to some extent have an statistical, rather than physiological basis relating to error in estimation of FFM using the SkF method: "measurement error will have the effect of reducing the slope of the regression line relating BMR to FFM and exaggerate any tendency for BMR/FFM to fall as weight increases".

Table 2.6. shows the coefficients of correlation between BMR/FFM with body mass and FFM.

As it may be noted, the fall in BMR/FFM was practically the same when FFM was measured either by SkF or D-UWW; therefore, the error in SkF measurement is not the reason to explain the decrease in BMR/FFM from light to heavy individuals. It was TBK the method that gave the highest fall. Probably TBK is associated with a higher measurement error than D-UWW & SkF; however, it would have to be too large to account for the difference in BMR/FFM between light and heavy subjects.

Errors in the estimation of FFM of different methods may not be an explanation for this negative relationship, but as it has been said, ratio standards overscale by converting a positive relation into a negative one and therefore, their use should be avoided.

A possible physiological explanation for this finding has been that, perhaps, heavier individuals are more muscular than their lighter counterparts relative to their B.M. (Lawrence et al, 1988) and so the BMR/FFM would be lower for heavier individuals because of the low metabolic activity of muscle mass. This possibility shall be discussed below.

For the sake of simplicity only the FFM estimation by densitometry (D-UWW) shall be used to express BMR per kg of FFM.

Linear Regression. The regression of BMR [kcal/day] on FFM [kg] was found to be different for each sex (figure 2.6.):

FEMALES (n = 78) BMR = 243.26 ($\pm$ 114.2) + 24.65 * FFM ($\pm$ 2.6);
r^2 = 0.55; r = 0.74; RSD = 113.02

MALES (n = 78) BMR = 695.74 ($\pm$ 165.3) + 18.2 * FFM ($\pm$ 2.68);
r^2 = 0.38; r = 0.62; RSD = 170.93

BMR/FFM	FEMALES r = p =	MALES r = p =
on B.M.		
Skinfolds	(n=79) -0.12 n.s.	(n=78) -0.33; 0.003
	(n=27) -0.17 n.s.	(n=38) -0.42; 0.008
T B Potassium	(n=27) -0.13 n.s.	(n=38) -0.41; 0.01
Densitometry	(n=78) -0.16 n.s.	(n=78) -0.35; 0.002
	(n=27) -0.07 n.s.	(n=38) -0.50; 0.002
on FFM		
Skinfolds	(n=79) -0.23; 0.04	(n=78) -0.37; 0.001
	(n=27) -0.23 n.s.	(n=38) -0.45; 0.005
T B Potassium	(n=27) -0.40; 0.04	(n=38) -0.59; 0.0001
Densitometry	(n=78) -0.25; 0.03	(n=78) -0.43; 0.0001
	(n=27) -0.23 n.s.	(n=38) -0.55; 0.0004

Table 2.6. Relationship between (BMR/FFM) with body mass and with FFM estimated by different methods.

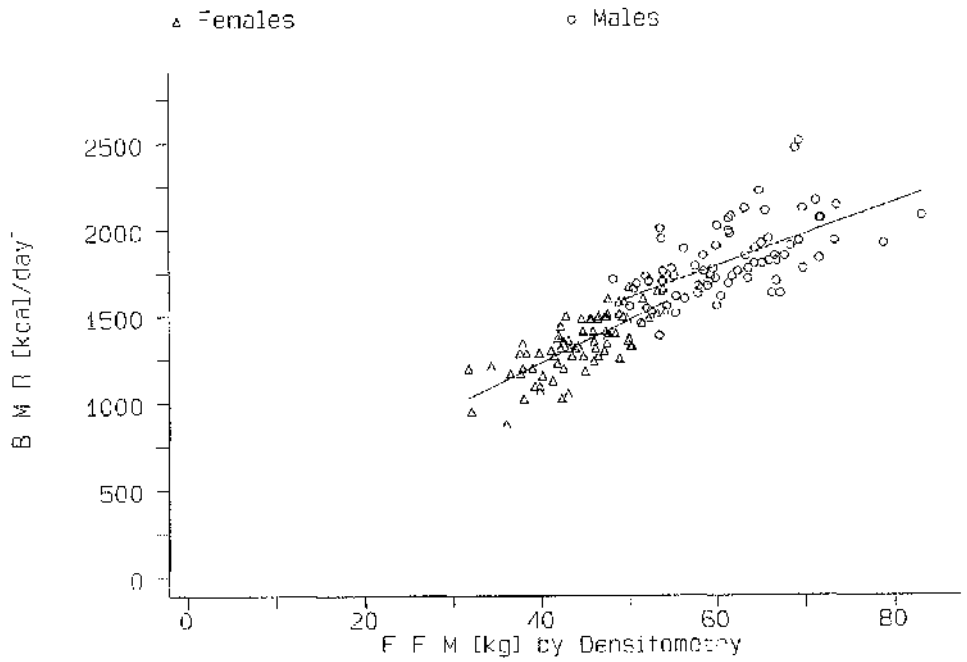

Figure 2.6. Regression of BMR on FFM for females (n=78) and for males (n=78).

For females 55% of the BMR variance was explained by FFM and for males 38%.

Comparing these analyses from those using B.M. it can be appreciated that for females B.M. explained 48% of the BMR variability and the RSD was 120.4 kcal/day, so there is some advantage in using FFM over B.M.. For males, B.M. explained 39% and the RSD = 167.5 kcal/day, so the prediction is slightly better using B.M., but practically the same.

When slopes and coefficients were tested by ANOVA, it was found that constants were significantly different between sexes (p=0.03) but slopes were not (p=0.11). When the same slope was allowed the constant term became more significantly different between sexes (p=0.0011). The equations obtained were (r^2 = 0.78; RSD = 145.66 kcal/day) BMR [kcal/day] =

FEMALES (n = 78) 436.44 (± 84.9) + 20.3 * FFM (± 1.87);
RSD = 114.4 kcal/day
MALES (n = 78) 567.75 (± 116.2) + 20.3 * FFM (± 1.87);
RSD = 170.5 kcal/day.

The fact that no statistically significant different slope was found between sexes may indicate that per kg of FFM the E.E. is the same but that there are other factors responsible for BMR variation among sexes.

Comparing the F values between the ANOVA of BMR on B.M. and BMR on FFM it was found that the effect of sex in the first instance presented a F value of 30.5 and in the second of 13.3, indicating that per kg of FFM there was a lower sex effect than per kg of B.M.. The RSDs about the line of best fits (114 and 171 kcal/day for females & males) indicated however, that in relation to the FFM there was still considerable variation in the BMR of individual subjects.

Simple Ratio Standard (BMR on FFM). The ratio standards or constant multipliers of the FFM were (r^2 = 0.99; RSD = 156.4): BMR [kcal/day] =

FEMALES (n=78) 30.05 * FFM (± 0.29); RSD = 115.6
MALES (n=78) 29.4 * FFM (± 0.35); RSD = 188.6

These ratio standards were not significantly different between sexes (p=0.18) and the equation obtained for both sexes is (n=156 r^2 = 0.99; RSD = 156.8): BMR [kcal/day] =

29.62 * FFM (± 0.23); RSD = 156.8 kcal /day

The use of ratio standards should be avoided, as was in the case of B.M., because the CV_x/CV_y did not equal the coefficient of correlation "r", i.e.,

Females: CV_x / CV_y: 11.34/12.51 = 0.92 and r = 0.74, and

Males: CV_x / CV_y: 11.86/11.88 = 1.0 and r = 0.615.

Power Function Ratio Standards (P.F.R.St.). The ANOVA showed the power function parameter to be not significantly different between sexes (i.e. 0.719; p = 0.12).

The ANOVA of BMR on $FFM^{.72}$ showed the slopes or constant multipliers to be different for each sex ($p<0.0001$). The data was then analysed allowing a different constant for each sex but a common power function parameter (Figure 2.7.). The resulting power function equations were (r^2 = 0.99 RSD = 144.98): BMR [kcal/day] =

FEMALES (n=78): $87.6(\pm 0.84) * FFM^{.72}$; RSD = 113.33

MALES (n=78): $93.9(\pm 1.0) * FFM^{.72}$; RSD = 170.99

The best model may be considered the one that gave the least error of prediction.

	Females	Males	Both
Simple Ratio	115.6	188.9	156.4
Linear			145.7
Regression	114.4	170.5	
Power Function	113.3	170.9	145.5

The error of prediction was greatest by the simple ratio standard model and the other two models predicted practically equal. In theory, the relationship between BMR and FFM is more likely to be a power function rather than a linear model for a wide range of body fat free masses and that would make the use of the P.F.R.St. model to be more recommended. However, different figures have been found between groups of subjects, as the study of Lawrence et al, 1988 and the present one. Then, the advantage of using the P.F.R.St. model over the linear regression model would be questionable.

Relationship between BMR expressed per kg of FFM [kcal/kg] with body mass and with FFM. A negative correlation was found between the expression BMR/FFM with B.M., which was not significant for females (r=-0.16; p=0.15) but significant for males (r=-0.35; p=0.0015); when BMR/FFM was correlated versus FFM, the relationship was significant for both sexes (Females r=-0.25, p=0.03; Males r=-0.43, p=0.0001).

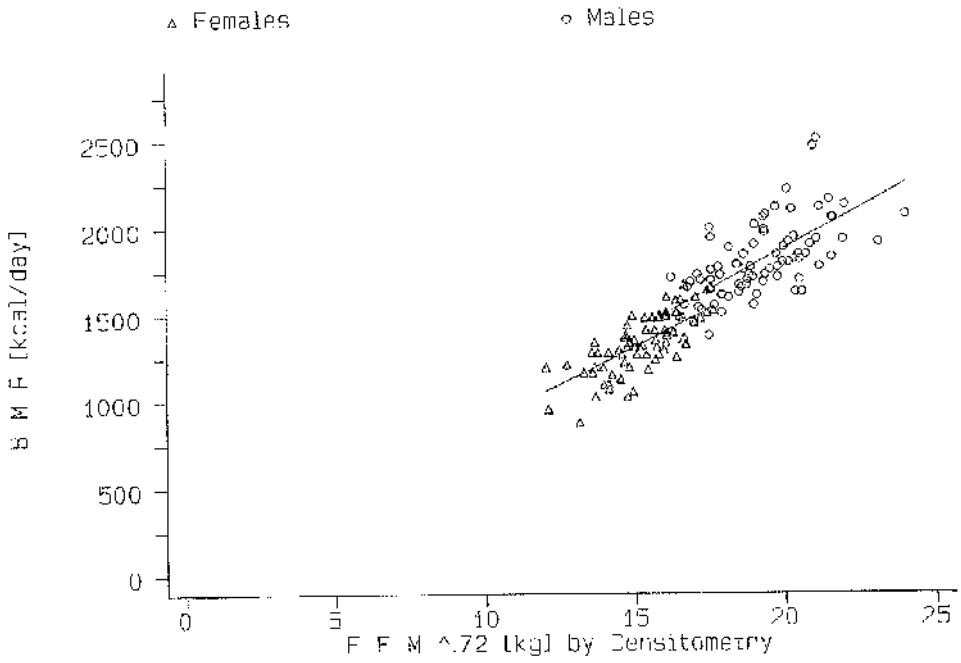

Figure 2.7. Power function ratio standard of BMR on FFM for females and for males.

This negative relationship was overcome by the use of the P.F.R.St.. When correlated on B.M.: for females the r value only changed from negative to positive but still not significant: r=0.16; p=0.14 and for males r=-0.06; p=0.63. When correlated on FFM: females r=0.12; p=0.3 and males r=-0.12; p=0.3.

Even though there is a negative and significant relationship between BMR expressed per kg of FFM the values for "r" are very low and probably imply that although statistically significant the practical importance is rather small. This relationship would most probably be important in groups of individuals with a greater FFM range than the one of this study.

Variation of BMR with fat free mass. For a given value of FFM ± 2.5 kg (i.e., each 5 kg) there was found considerable BMR variability; table 2.7. shows the BMR and the BMR per kg of FFM and the Cs.V. of both expressions.

Large mean Cs.V. were found when BMR was expressed per kg of FFM; Cs.V. were of 12.5% for females and 11.9% for males. For the FFM sub-groups the C.V. ranged from 7.7 to 13% for females and for males it ranged from 6.2 to 12.6% and not particular trend was found. When standardized for FFM the Cs.V. of the FFM subgroups were virtually the same, but lower for the groups of females and males.

Within FFM sub-groups, BMR showed no significant difference in all but in the 50-54.9 kg female sub-group (p=0.01). This sub-group was formed by 9 women, with BMR values not significantly different to the whole group but with a fairly constant trend to increase their BMR as FFM did. When the FFM range was increased to 7 kg, i.e., from 48-54.9 kg (n=19), the within group BMR variability became not significantly different (p=0.08).

One-way analyses of variance were performed to compare the BMR between sub-groups of FFM and showed, for females a significant difference (p<0.0001), 7 out of 10 comparisons presented significant difference. For males, the analysis also showed a significant difference; however most comparisons (21 out of 28) between sub-groups were not significantly different.

No relationship was found between BMR variability and FFM values.

Groups by FFM	Females n =	Females BMR BMR/FFM X ± S.D. (range)	Females C.V. [%]	Males n =	Males BMR BMR/FFM X ± S.D. (range)	Males C.V. [%]
30-34.9	3	1118 ± 145.7 (950-1210)	13.0	-	-	-
		34.2 ± 4.10 (29.7-37.7)	12.0			
35-39.9	13	1159 ± 127.4 (878-1339)	11	-	-	-
		30.3 ± 3.31 (24.4-35.4)	10.9			
40-44.9	25	1292 ± 121.4 (1022-1498)	9.4	-	-	-
		30.3 ± 2.77 (24.2-35.0)	9.1			
45-49.9	28	1434 ± 110.8 (1238-1670)	7.7	3	1555 ± 158.4 (1397-1714)	10.2
		30.1 ± 2.22 (25.6-33.6)	7.4		31.9 ± 3.40 (25.9-25.6)	10.7
50-54.9	9	1502 ± 121.8 (1325-1656)	8.1	15	1669 ± 166.6 (1382-2002)	10.0
		28.8 ± 1.79 (26.3-31.0)	6.2		31.6 ± 3.02 (25.9-37.4)	9.6
55-59.9	-	-	-	18	1706 ± 107.2 (1512-1901)	6.3
					30.3 ± 2.84 (26.7-34.2)	6.2
60-64.9	-	-	-	16	1890 ± 177.3 (1613-2218)	9.4
					30.3 ± 2.84 (26.7-34.2)	9.4
65-69.9	-	-	-	18	1925 ± 242.6 (1627-2506)	12.6
					28.6 ± 3.34 (24.2-36.2)	11.4
70-74.9	-	-	-	6	2028 ± 126.0 (1829-2160)	6.2
					28.1 ± 1.80 (25.6-30.3)	6.4
ALL FEM. 31.7-54.1	78	1338 ± 167.5 (878-1670)	12.5	-	-	-
		30.2 ± 2.73 (24.2-37.7)	9.0			
ALL MALES 48.1-83.1	-	-	-	78	1814 ± 215.5 (1382-2506)	11.9
					29.7 ± 3.02 (24.2-37.4)	10.2

Table 2.7. Variation of BMR [kcal] with fat free mass [kg].

Summarizing, equations are given to predict BMR from B.M.; the linear regression analysis (LRA) and the power function ratio standard (PFRSt) models gave similar RSDs and were lower than the simple ratio standard (SRS) model. B.M. may explain 55% of females and 38% of males BMR's variance. The C.V. of the BMR was of about 12% for each sex, and it ranged from 7 to 14% for females and from 6 to 13% for males, standardization for B.M. gave very similar figures within groups. The variation of BMR with B.M. subgroups showed that within groups, there were not significant differences but in a male's subgroup. Between groups differences were all significant.

For the estimation of BMR from FFM, it was found that most of the variation in the estimation of BMR per kg of FFM is caused by differences in the estimation of FFM assessed by different methods; once BMR is expressed per kg of FFM estimated by any one of the methods analysed the differences between sexes became almost nil.

The constant multipliers obtained by both the LRA and the PFRSt models were shown to be not significantly different between sexes. However, the low level of explanation of BMR variance (55% for females and 38% for males) showed that there are other factors responsible for the remaining variance.

The LRA model presented the least RSD followed by the PFRSt which gave a very similar RSD and lastly the SRS model.

The analysis of the variation of BMR with FFM showed the C.V. to be of about 12% for each sex. Within FFM subgroups, each 5 kg, the Cs.V. ranged from 8 to 13% for females and from 6 to 13% for males and standardization for FFM gave very similar figures; there were not significant differences in the BMR but in one of the females subgroups. Between subgroups, there were significant differences.

There was found a tendency of the BMR expressed per kg of FFM to fall as the FFM increased which was higher in males. This negative relationship has been overcome by the use of the PFRSt.

2.3. B M R variability explained by total body Potassium as an index of muscularity.

It has been reported that the variability of the BMR may be explained by variations in age, sex, B.M., FFM and its composition, within subject variability, sex, ethnicity and error of the methodology employed, among the main variables.

The fact that BMR variation is considerably reduced when the size of FFM is taken into account, has led to the widespread use of FFM as a metabolic reference standard. However, as it has just been seen, at a given FFM, BMR varies as much as with B.M. between

individuals; this observation has also been found by Ravussin et al, 1986 and Lawrence et al, 1988.

One of the problems in estimating B.C. by some of the most used methods, i.e., body density, total body water & total body potassium, is the assumption of a constant composition of the FFM. Also, the prediction of BMR assumes in some instances that per unit of FFM, M.R. (metabolic rate) is constant.

Differences in the relative contributions to the fat free compartment of tissues such as skeleton, skeletal muscle and viscera have been suggested as important contributors to the BMR variation.

Total body potassium (TBK) is a component of muscle mass and it may be used to estimate the extent to which the degree of muscularity can further explain BMR variability.

Twenty seven females and 38 males had their TBK measured and the analyses of these data was sought to explore how much of the remaining variance of BMR, predicted from B.M. or from FFM, could be explained by TBK.

Females. In those females that had their TBK measured (n=28), B.M. explained 46.5% of BMR variability, RSD = 106.1 kcal/day, when TBK was included in the prediction a further 10% was explained, i.e., 57.8%, RSD = 95.0 kcal/day, still remaining 42% to be explained by other factors. However, when B.M. and TBK were both put in the same regression the constant term became not significant and when it was excluded, the r^2 became = 0.995 and the RSD = 95.97 kcal/day, the obtained equation is:

$$BMR\ [kcal/day] = 12.25*B.M.\ (\pm 3.60) + 0.223*TBK\ [mmol]\ (\pm\ 0.069)$$

TBK on its own explained 51.5% of BMR variance, RSD = 99.9 kcal/day.

$$BMR\ [kcal/day] = 492.6\ (\pm\ 166.8) + 0.294\ *\ TBK\ (\pm\ .056)\ [mmol]$$

The prediction of BMR using FFM presented a $r^2 = 0.551$ and a RSD = 97.6 kcal/day, when TBK was included in the regression analysis the r^2 increased to 0.574 and the RSD diminished to 97.2 kcal/day, but both terms became non significant in the regression equation. Then, TBK added nothing to FFM prediction.

Males. Thirty eight males had their TBK measured, in these subjects B.M. explained 33% of BMR, RSD = 165.2 kcal/day, a further 5% was explained by TBK, i.e., 38%, RSD = 161.1 kcal/day. Age

increased the prediction a further 10%, i.e., to 48.5%, RSD = 149 kcal/day. If only B.M. and age were included for the regression analysis, age increased the prediction above B.M. by 15%, RSD = 147.5 kcal/day; the inclusion of TBK after age did not have any significant effect on the prediction.

TBK on its own explained 36% of BMR variance with a RSD = 161.4 kcal/day; as in females, better than B.M. alone.

$$BMR = 907.4\ (\pm\ 206.3) + 0.219 * TBK\ [mmol]\ (\pm\ 0.049)$$

The regression of BMR on FFM presented a $r^2 = 0.355$ and a RSD = 162.04 kcal/day, when TBK was included in the regression analysis the r^2 increased to 0.373 and the RSD did not change, and both terms became non significant in the regression equation. Then, TBK did not add anything to FFM prediction.

Relationship between BMR/FFM and TBK. The fact that BMR/FFM diminished from light to heavy individuals, made look out for physiological facts that could explain the reason. A possible explanation would be the proportion of skeletal muscle, i.e., if heavier individuals were more muscular.

In this study it was found a positive and highly significant relationship between TBK with both B.M. (r=0.96) & FFM (r=0.87). Indirectly indicating that heavier individuals have more muscle.

Skeletal muscle is a low metabolic contributor to the BMR as it is not used during this measurement; therefore it would be expected an indirect relationship between BMR expressed per kg of FFM with TBK.

Results:

For females, the relationship between BMR/FFM on TBK was not significant, i.e., r=-0.08; p=0.69. Reasons were looked for and it was interesting to find that for those 28 females that had their TBK measured, there was a weak & not significant negative relationship between BMR/FFM with FFM (r=-0.23; p=0.26). Probably showing that this group had very similar amounts of FFM and of muscle.

For males, the correlation between BMR/FFM on TBK was r = -0.45; p = 0.0045 and the 38 males that had their TBK measured, had a correlation of BMR/FFM on FFM r = -0.55; p = 0.0004.

Then, at least in males, the decrease in BMR/FFM could in part be explained by a greater amount of TBK which represents a higher muscle mass and so differences in the composition of the FFM due to

the relative proportions of 'active' organs compared to 'inactive' organs, might be the reason for the lower M.R. per kg of FFM as mass increases.

2.4. BMR variation explained by other variables.

Univariate correlations were performed between BMR and the B.C. variables of females and males and are presented in table 2.8.

The results showed that there was not a significant correlation between BMR and age for females but significant for males, although weakly. Individual skinfolds (SkF) were not included because all were n.s., the exception being the triceps SkF for females. All other variables but the sum of 4 SkF (Σ4SkF) for both sexes and biestileon diameter for females were positively correlated with BMR. B.M. & FFM were the variables that most explained BMR, followed, for females, by the sum of 3 circumferences (Σ3circ) and, for males, the biacromial diameter & the sum of 4 bone diameters (Σ4diam). Calf circ. presented good correlation in both sexes.

Stepwise multiple regression analyses (Stpw. mult. regr. an.) were performed to find the best predictors of BMR. The following variables, among other, were included: B.M., $BM^{\cdot 69}$, FFM, TBK, arm, tight and calf circs and its sum, arm muscle area (AMA), each of 4 bone diam. and its sum, each of 4 SkF and its sum, and BMI.

Females (n=27): The best and unique chosen variable was FFM explaining 55.3% of BMR, with a RSD = 97.6 kcal/day and it was shown that the constant term was not significant (p = 0.21) and when it was excluded, FFM alone on these 27 females explained 99.5% of BMR with a RSD = 95.84 kcal/day. The obtained equation is:

$$BMR\ [kcal/day] = 29.73\ (\pm\ 0.413) * FFM$$

VARIABLES	FEMALES (n = 79) r = ; p =	MALES (n = 78) r =; p=
AGE [years]	0.06; n.s.	-0.25; 0.03
BODY MASS [kg]	0.70; 0.03	0.62; 0.0001
HEIGHT [cm]	0.57; 0.0001	0.40; 0.003
B M I [kg/m^2]	0.48; 0.0001	0.38; 0.0006
Body fat [%] (D)	0.04; n.s.	0.01, n.s.
FFM [kg] (D)	(n=78) 0.74; 0.0001	0.62; 0.0001
TRICEPS SkF	0.29; 0.01	0.1; n.s.
SUM 4 SkF	0.16; n.s.	0.08; n.s.
ARM CIRCUMFERENCE*	0.45; 0.0001	0.40; 0.0003
WAIST CIRCUMFERENCE	(n=61) 0.61; 0.0001	(n=45) 0.31; 0.037
BUTTOCKS CIRC.	0.59; 0.0001	0.46; 0.0001
TIGHT CIRC. *	0.60; 0.0001	0.49; 0.0001
CALF CIRCUMFERENCE*	0.58; 0.0001	0.54; 0.0001
SUM 3 CIRCS. *	0.63; 0.0001	0.53; 0.0001
BIACROMIAL DIAMETER	0.41; 0.0002	0.53; 0.0002
BIILIAC DIAMETER	0.28; 0.0067	0.37; 0.0009
BIESTILEON DIAMETER	0.14; n.s.	0.38; 0.0017
FEMORAL DIAMETER	0.29; 0.010	0.44; 0.0001
SUM 4 DIAMETERS	0.49; 0.0001	0.55; 0.0001
TB POTASSIUM [mmol]	(n=28) 0.72; 0.0001	(n=38) 0.60; 0.0001

Table 2.8. Univariate correlations for BMR with all variables studied. Correlation coefficient (r) and statistical significance (p).

* circumferences that were included for the sum of 3 circs.

As TBK was not among the chosen variables, it was excluded from the Stepw. mult. regr. an. and in this way the number of volunteers included increased from 28 to 78. Again, FFM was the first chosen predictor, explaining 54.5% of BMR variability, RSD = 113.0 kcal/day. AMA further increased the prediction to 58.3%, RSD = 109.8 kcal/day, and the Σ3circ to 62.6%, RSD = 104.6 kcal/day. Both AMA and Σ3circ can be assumed as indirect muscular indicators in lean subjects and so, it can be deduced, that indirect muscularity indexes were important explanatory variables of BMR variation in these females. As the constant term was not significant it was excluded from the regression and without it the RSD was 104.7 kcal/day. The obtained equation is: BMR [kcal/day] =

$$25.1*FFM\ (\pm\ 3.6)\ -\ 9.4*AMA\ (\pm\ 3.0)\ +\ 4.4*\Sigma 3circ\ (\pm\ 1.3)$$

It was interesting that TBK was not able to explain any further the remaining variance after FFM in those females that had their TBK measured (n=27) and when all females were integrated (n=78) two indirect muscularity indexes were able to explain a further 8% of the remaining variance. However, the S.D. about the best fits (98 kcal/day for those 27 females and 105 kcal/day for all 78) indicated that either way there is still considerable variation in the BMR of individual women. Probably, in practical terms the reduction from 113 kcal/day, including only FFM, to 105 kcal/day, adding AMA and Σ3circ, is not too important.

FFM was excluded, because of the practical problems related to its measurement and accurate prediction; $BM^{.69}$ was the following best BMR predictor variable (r^2=0.48) and as the constant term was not significant (p=0.52), it was excluded and $BM^{.69}$ explained 99.2% of BMR variation and the RSD = 120.53 kcal/day; no other variable increased any further the prediction; the obtained equation is:

$$BMR\ [kcal/day]\ =\ 85.6\ (\pm\ 0.871)\ *\ BM^{.69}$$

which is equal to the power function ratio standard.

B.M. & the suprailiac SkF (supil-SkF) and equally B.M. & the calf circumference (calf-circ) were the following best chosen predictors, excluding $BM^{.69}$, both combinations achieving about 51% of explanation of BMR variability and a RSD = 118 kcal/day. A noteworthy observation was that when calf-circ was included in the analysis, the constant term became non statistically significant, making the calf-circ a better predictor than the supil-SkF. The

regression of BMR on B.M. & calf-circ was then performed using the no-constant option and the RSD was 117.8 kcal/day and the r^2 = 0.993. The obtained equation is:

BMR [kcal/day] = 14.5 * B.M. (± 2.79) + 15.4 * calf-circ (± 4.44)

The best variables which could explain TBK were also looked for to study whether their inclusion in the regression analysis would increase the prediction. It was interesting to find that SkFs, which are used as indicators of body fat, predicted TBK to a better extent than body girths and AMA which are used as indicators of muscularity.

Males (n=38): When 'Stpw. mult. regr. an.' was performed to predict BMR from B.C. variables, the same as those for females; the Σ4diam was the first explanatory variable, explaining 39.9% of BMR variability, RSD = 156.5 kcal/day, then age increased the prediction to 47.7%, RSD = 148 kcal/day, and TBK to 53.7%, RSD = 141.3 kcal/day.

It was note-worthy that bone diameters, more likely to be indirect anthropometric indicators of the skeleton, in this group of males (n=38), explained 40% of BMR variability; age and TBK further increased the prediction by 8% and 7% respectively. Also that whenever the Σ4diam was part of BMR prediction equations, the constant term became not significant and when it was excluded from the analysis, the best equation explained 99.5% of BMR variability with a RSD = 140.4 kcal/day and is as follows: BMR [kcal/day] =

17.3 * Σ4diam (±2.8) - 6.2*age (±2.7) + 0.13*TBK [mmol] (± 0.05)

Because of the difficulty to carry on the TBK measurement and the small number of subjects that had it measured, indirect anthropometric indicators of TBK that could alternatively explain BMR variability were looked for. When TBK was excluded the number of cases increased from 38 to 78 males; it was found that the first chosen variable was B.M. explaining 38.9% of BMR variation with a RSD = 169.5 kcal/day, then age (48.3%, RSD = 157 kcal/day) and lastly the Σ4diam increased the prediction to 52.3%; RSD = 152 kcal/day. In the presence of the Σ4diam, the constant term became not significant and excluding it, the 3 variables explained 99.3% and the RSD without this term yielded 150.9 kcal/day and the equation has the following form: BMR [kcal/day] =

12.15*BM (±2.71) - 8.67*age (±2.14) + 14.27* Σ4diam(±2.32)

When the Stepw. mult. regr. an. was repeated with the no-constant option, $BM^{.69}$ was first chosen, instead of B.M., explaining 99.2% of BMR variability with a RSD = 169.1 kcal/day; age was also included, increasing the prediction to 99.3 % with a RSD = 157.9 kcal/day and lastly the Σ4diam was included increasing the prediction to 99.3% with a RSD = 152.1 kcal/day. The obtained equation is: BMR [kcal/day] =

$$89.4 * BM^{.69} (\pm 16.1) - 8.78*age (\pm 2.2) + 9.3 * \Sigma 4diam (\pm 3.5)$$

Compared to females; for males there were another variables besides $BM^{.69}$ that could explain more of BMR and FFM was not chosen among the explanatory variables.

The degree of prediction of BMR by either the two ways above described is practically the same.

Also studied was the effect that the best variables on each sex would have on the other sex, in order to find out whether the same anthropometric variables could be used for both.

The best predictor variables got for females (i.e., B.M. and calf-circ), were studied for males; it was found that these variables could explain 39.9% of BMR variability RSD = 169.3 kcal/day. Also, as in females the constant term was not significant, then it was excluded and age included because of its significant effect in males; the r^2 increased 0.992, because of the no-constant effect, and the RSD = 163.2 kcal/day. The obtained prediction equation is: BMR [kcal/day] =

$$12.92*BM (\pm 3.4) + 29.26*calf\text{-}circ (\pm 6.34) - 6.0*age (\pm 2.2)$$

The best predictor variables got for males (i.e., B.M., Σ4diam and age), were tried in females; these variables explained 50.3% of BMR, RSD = 119.7 kcal/day. The constant term and age were not significant and both were therefore excluded. The same level of prediction as using the best variables got for females, was achieved, i.e., 99.3% and a RSD = 119.9 kcal/day. The obtained equation is: BMR [kcal/day] =

$$16.34 * BM (\pm 2.55) + 5.51 * \Sigma 4diam (\pm 1.80)$$

Both sexes: When both sexes were analysed together by ANOVA (n=65), it was found that the slope of BMR on TBK was not significantly different between sexes (p = 0.40), but the constant term was different for each sex and B.M. inclusion was no further statistically significant (p = 0.092). Therefore the analysis was

performed allowing these facts. The following equations were obtained (r^2 = 0.779; RSD = 138.65 kcal/day): BMR [kcal/day] =

Females: 665.48 (± 57.4) + 0.2357 * TBK [mmol] (± 0.037)
Males: 836.39 (± 156.4) + 0.2357 * TBK [mmol] (± 0.037)

However, when age was included among the variables, TBK became non significant (p = 0.063). Thus, showing that B.M. accompanied by age were better BMR predictors than a FFM component; probably FFM variation among subjects is, on the whole, better explained by the aging process. However, it must be said that age alone showed to be not significantly related to BMR in females; for males age explained 6.2% of the variance (p = 0.03) and when both sexes were analysed together, age was significant. The obtained final equation (r^2 = 0.792; RSD = 140.84): BMR [kcal/day] =

Females: 519.33 (± 30.54) + 17.42*BM (± 1.54) - 5.50*age (± 1.49); RSD = 121.35

Males: 759.56 (± 111.7) + 17.42*BM (± 1.54) - 5.50*age (± 1.49); RSD = 156.51

BMR variation explained by age.

Independent variables	Females (n = 79) (r = ; p =)	Males (n = 78) (r= ; p=)
Body mass [kg]	0.03; n.s.	0.09; n.s.
Height [cm]	-0.06; n.s.	-0.14; n.s.
Fat %	0.07; n.s.	0.31; 0.006
FFM (UWW) [kg]	0.005; n.s.	-0.02; n.s.
BMR [kcal/day]	-0.06; n.s.	-0.25; 0.03
TBK [mmol]	-0.09; n.s. (n=28)	0.11; n.s. (n=38)

Table 2.9. Age effect on body composition variables and BMR.

None of the studied variables were affected by age in females.

In males, there was a slightly higher effect of age on some of these variables. BMR decreased as age did, by a factor of 6.65 kcal per year, or about 4% per decade; it was important to find that there were two 18 year old males with the highest BMR values that played an important effect in making BMR to diminish as age did, when this two subjects were eliminated, age became not significant (n=76; r=-

0.19; p=0.09). However, age reminded a significant variable to predict BMR in males, even after B.M., FFM and other variables had been taken into account. Also significant was the direct relation with fat% and aging, but fat% was not significant for BMR prediction. Then, despite the small sample of this study, it would be recommended the use of decade periods instead of the larger periods suggested by FAO/WHO/UNU, 1985.

2.5. Comparison between BMR measured and calculated with various predictive equations.

Table 2.10. shows the comparison between BMR measured and calculated with the equations proposed by: FAO/WHO/UNU, 1985; Kleiber, 1947; Dubois & Dubois, 1916; Harris & Benedict, 1919 and Cunninham, 1980.

Mean differences were all within ± 10%, even taking into account the 95% C.I. for the mean difference. However, variations around mean differences were large, the limits of agreement reached differences up to nearly ±25% for both sexes. Females' calculations showed a bias to overestimate BMR while males' calculations to underestimate the BMR measured value.

FAO/WHO/UNU mean estimations were the ones that were most near to the mean measured values for females i.e., -1.7% or 23.4 kcal/day, thus resulting in a non significant statistic (p=0.1); however, the limits of agreement showed a similar range to the other equations. The exception was Cunningham's prediction that was clearly biased towards positive values, or to overestimate BMR related to the measured.

Dubois' and Cunninham's mean predictions were most near to the mean measured BMR for males, ie, -0.4 and -0.6% respectively or -7 and -13 kcal/day, n.s.; the rest of the predictions were around 5% below that measured (p = 0.0001); however, the same as with females, the limits of agreement of all predictions were more or less in the same range.

These equations should not be used for individuals, as big mistakes can be done. Probably, FAO/WHO/UNU, 1985 equation for females and Du bois equation for males may be recommended, but at the light of the variances of all equations it is really difficult to say which equation correctly predicts BMR.

Method	Mean ± SD (min-max) [kcal/day]	DIFFERENCE		
		Mean ± SD (min-max) [% mrd.]	95% C.I. For mean difference [% mrd.]	Limits of Agreement (X± 2 S.D.) [% mrd.]
Measu-red	1338± 166.4 (878-1670)	-	-	-
W H O	1315± 96.5 (1087-1631)	-1.7 ± 9.58* (-19.5 to 20.7)	-3.8 to 0.5	-20.9 - 17.5
Klei-ber	1399± 120.7 (1127-1614)	4.3 ± 8.80 (-12.6 to 25.1)	2.3 to 6.3	-13.3 - 21.9
Du-bois	1388± 105.2 (1135-1659)	3.6 ± 8.95 (-11.6 to 25.2)	1.6 to 5.6	-14.3 - 21.5
Harris & Bend	1368± 80.6 (1201-1606)	2.3 ± 9.47 (-17.9 to 28.4)	0.2 to 4.5	-16.6 - 21.3
Cunnin -gham	1461± 108.9 (1186-1669)	8.5 ± 8.03 (-5.8 to 31.2)	6.7 to 10.3	-7.6 - 24.6

Table 2.10.A. Comparison between BMR measured and estimated with various predictive equations for females (n=78).

* $p > 0.1$, n.s. by "Student's paired t test".

Method	Mean ± SD (min-max) [kcal/day]	DIFFERENCE		
		Mean ± SD (min-max) [% mrd.]	95% C.I. For mean difference [% mrd.]	Limits of Agreement (X± 2 S.D.) [% mrd.]
Measu-red	1814± 215.5 (1382-2506)	-	-	-
W H O	1717± 120.4 (1495-2090)	-5.6 ± 9.41 (-28.7 to 12.5)	-7.7 to-3.5	-24.4- 13.2
Klei-ber	1743± 151.3 (1440-2103)	-4.1 ± 8.78 (-27.0 to 14.8)	-6.1 to-2.2	-21.7- 13.4
Du bois	1820± 144.0 (1525-2244)	0.4 ± 8.52* (-21.1 to 17.5)	-1.5 to 2.3	-16.7- 17.4
Harris & Bend	1732± 142.7 (1434-2072)	-4.7 ± 8.88 (-27.3 to 13.7)	-2.7 to-6.7	-22.5- 13.1
Cunnin gham	1827± 173.3 (1531-2212)	0.6 ± 9.24* (-25.4 to 16.6)	2.7 to -1.5	-17.9- 19.1

Table 2.10.B. Comparison between BMR measured and estimated with various predictive equations for males (n=78).

* p > 0.5, n.s. by "Student's paired t test".

The results of this study have shown that although the slope of the equation relating FFM to BMR is not significantly different between sexes, the constant term did differ. Cunningham's predictive equation does not differ between sexes and Owen et al, 1987 found that the relationship between BMR and FFM was statistically indistinguishable between sexes.

2.6. General Discussion on BMR.

Large variations in BMR have been tried to be reduced by the use of FFM, and the remaining variation has been left, among other unknown factors, to the composition of the FFM, so that subjects with greater proportions of highly active organs related to those less active organs, will have greater BMR's.

In the present study, it was observed that for those females that had their TBK measured (n=27) FFM and no other variable explained most of BMR variance, to the point that the constant term became not significant. However, for all women together (n=78), AMA and the sum of 3 circumferences further increased the prediction. Probably those women that performed the TBK study were more homogeneous than the whole group. Other possibilities were $BM^{.7}$ and B.M. together with calf circumference; the three options gave similar prediction errors.

For males, the sum of 4 diameters and age were predictors that accompanied either TBK (for those that had it measured), $BM^{.7}$ or B.M.; all predictions giving practically the same residual standard deviations. It was interesting to find that FFM was not among the variables that could explain more of BMR variance in males. Reasons were looked for and it was found that there were two 18 year old males, both with the highest BMR values (around 2500 kcal/day), not explained by the FFM (about 69 kg), nor by the body mass (83 kg) values, and not so lean (about 17% fat), body mass and age (because they were amongst the youngest of the group) could explain more of BMR variance (r^2 = 0.483; RSD = 157.0 kcal/day). When these 2 subjects were excluded from the stepwise analysis, FFM became the variable that explained most of BMR (r^2 = 0.387 RSD = 148.2 kcal/day) and age was still significant but to a lower degree and the RSD was lower than when B.M. and age were the predictor variables (r^2 = 0.428; RSD = 144.1 kcal/day).

Either way, it may be noticed that the part explained either by B.M. or FFM and age did not even reach 50% of BMR variance in males and in females FFM explained about 55%. Also found was that at a given B.M. or FFM value there was considerable variation in the BMR of individual women and men. This might be explained since this was

a fairly homogenous group and it has been found by other investigators (Lawrence, 1988) that different to what happens in heterogenous groups where FFM explains over half of BMR variance, in homogeneous groups lower correlations are found.

A practical implication with the low levels of prediction achieved is that when BMR estimations are employed there shall be those subjects with real very low and those with real very high BMRs and an important point to be discussed is whether those subjects with low metabolic rates will put on weight or else will have to refrain from eating to be in energy balance. And the question arises: did obese persons had a low metabolic rate before they become obese? and, does the BMR of people in a low energy regimen becomes lower because of the food shortage, as a defence mechanism?. The answers to these questions are uncertain but something has been shown: those subjects with low metabolic rates compared to those with high metabolism, adjusted for age, sex and differences in FFM, have greater risk of gaining weight (Ravussin et al, 1988).

In this study it was found that FFM predicted BMR slightly better than B.M. in females and practically equal in males. It could have been expected that FFM could be a better metabolic standard than B.M. because of differences in fat mass and the fact that FFM has been shown to eliminate differences between sexes, fat%, etc.. But more important than fat mass (%) or sex is the relative proportion of active and inactive organs that are not possible to separate with this study and with none of the studied variables.

Besides B.M. and FFM there were some other B.C. variables that increased BMR prediction. Those variables were: TBK, body girths and bone breadths, the first two are essentially providing an estimate of muscle mass and bone breadths of the skeletal frame, both components of the FFM. The increase from the prediction of B.M. or FFM alone in females is about an extra 8%, reducing the RSD by about 8 kcal/day, and in males about an extra 14%, reducing the RSD by about 18 kcal/day. But, probably, the most important fact is that the inclusion of variables such as the sum of 3 body girths and the sum of 4 bone breadths, made the constant term to become not significant and even though the RSDs were of about the same magnitude with and without the constant term, if it was not longer significant and it was excluded, the part explained by the used variables nearly reached a 100% of BMR explanation. This fact makes wonder whether BMR variance may almost be entirely explained by variations in the composition of the FFM, although TBK and body girths as indirect muscularity indices and the sum of 4 bone breadths as indirect skeleton index, which make up more than half of the weight of the FFM but have a low resting metabolism and account to less than 25% of the

BMR (Brozek & Grande, 1955), were not the best estimators. Then, it would be needed to measure the mass and metabolic activity of those high metabolic active organs such as the liver, heart, lungs and kidney, which although in terms of mass they make up only about 6% of the FFM, they are responsible for about 60% of the BMR (Brozek & Grande, 1955), where at least part of the explanation of inter-individual BMR variation will lie. Relatively subtle variations in the relative proportions of active compared to those less active tissues therefore, could potentially have a major impact on BMR. Evidence that this could be the case in animals has been put forward by Koong & Ferrel, 1990 who observed that up to 40% of BMR differences between animals of the same weight and age but who had been subject to different nutritional regimes could be almost entirely explained by differences in the mass of the metabolically active organs making up body mass.

The use of B.M. in preference to FFM might be argued; the ease and accuracy with which B.M. can be measured and the measurement errors of the FFM (experimental and for the assumptions) makes B.M. to be preferred above FFM. Any error in the measurement of the FFM will necessarily distort the true relationship between BMR and FFM introducing a degree of variation with technical rather than biological cause. More discussion on FFM prediction and its estimation from different methods is presented in the body composition chapter.

The decrease of BMR per kg of either B.M. or FFM from light to heavy individuals could be physiologically produced in this group of subjects if, as weight increased, the proportion of metabolically active tissues such as the liver, kidneys, heart and brain declined and concurrently the proportion of tissues with comparatively low metabolic rates increased. Lawrence et al, 1988 have suggested that perhaps the most likely difference would be the proportion of skeletal muscle. Those subjects of this study that were heavier had higher TBK, and fat%, thus were more muscular, and fatter, than their lighter counterparts.

In the group of males that had their TBK measured (n=38) it was seen that there was a significant decrease in BMR/FFM from light to heavy individuals and also as TBK increased, demonstrating that BMR/FFM decreased as they were more muscular. Unfortunately, those females that had their TBK measured (n=27) did not present this decline in BMR/FFM from light to heavy individuals; but, in the whole group (n=78) there was a significant decrease and AMA and the sum of

3 circumferences, two indirect variables of muscularity, accompanied FFM as the variables that best explained BMR.

Related to the non-fat adipose tissue which becomes a part of the FFM, the fatter the individuals were the greater the portion the FFM contained of this non-fat tissue. It has been proposed that this tissue might have a lower metabolic rate than the rest of the FFM, thus explaining the decline of BMR/FFM as subjects are fatter. However, none of the two sexes presented body fatness as a variable that would have an influence on BMR, not only when expressed per kg of FFM but neither when expressed per kg of B.M., nor when FFM nor B.M. were not included in the prediction. Then, the fat free component of adipose tissue should approximate to the average metabolic rate of the rest of the FFM or else it compensates with another tissue.

If normalization of data is what is wanted, it has been stated by Tanner, 1949, Nevill et al, 1992 and Winter, 1992 that either the regression standard or the power function ratio standard either of B.M. or FFM are the correct way for doing so. In the present study it was found that BMR is approximately constant when divided by a power function of either B.M. or FFM. As a reference standard it seems to be more appropriate to relate B.M. to a power function than to simply express metabolic rate per kg of B.M. or FFM.

It has been found by Valencia et al (1994) that BMR was significantly improved by combining FFM with BMI, in the present series it was not found so.

For females, FFM alone explained 55% and a RSD = 120.4 kcal/day of BMR variance and the inclusion of BMI did not further increase the prediction ($p=0.51$), ie, same r^2, and a little lower RSD, ie, 113.4 kcal/day.

For males, FFM on its own explained 37.8% of BMR variance with a RSD = 170.93 kcal/day; the inclusion of BMI after FFM did not have any effect on BMR prediction ($p=0.92$).

3. ENERGY COST (METABOLIC RATE) OF WALKING ON THE TREADMILL (TWMR).

3.1. Distribution of the data of TWMR.

VARIABLE	MEAN ± S.D. (MIN-MAX)	p 5%	p 25%	p 50%	p 75%	p95%
B M R [kcal/min]	0.93 ± 0.116 (0.61-1.16)	0.71	0.86	0.93	1.03	1.11
TWMR [kcal/min]	2.94 ± .375 (2.06-3.68)	2.26	2.72	2.98	3.14	3.63
BMR / BM [cal/min/kg]	16.8 ± 1.63 (13.4-20.6)	14.3	15.6	16.9	18.0	19.9
TWMR / BM [cal/min/kg]	53.23 ± 4.66 (46.6-66.9)	47.0	49.7	52.8	57.0	62.4
BMR / FFM [cal/min/kg]	21.0 ± 1.90 (16.8-26.2)	17.8	19.6	21.2	22.2	24.3
TWMR / FFM [cal/min/kg]	66.3 ± 6.40 (55.3-85.3)	58.4	61.5	64.5	70.3	79.2
TWMR/BMR	3.17 ± 0.262 (2.58-3.95)	2.75	3.01	3.16	3.32	3.68
$BMR/BM^{.68}$ $[cal/min/kg^{.68}]$	59.35 ± 5.41 (47.2-69.8)	50.0	56.1	59.4	63.6	68.3
$TWMR/BM^{.61}$ $[cal/min/kg^{.81}]$	114.0 ± 9.72 (100 -138.5)	101	107	113	120	135

Table 2.11.A. Distribution of the data of the subjects that performed the treadmill walking metabolic rate study. Females (n = 76).

VARIABLE	MEAN ± S.D. (MIN-MAX)	p 5%	p 25%	p 50%	p 75%	P95%
B M R [kcal/min]	1.26 ± .150 (.96-1.74)	1.05	1.16	1.23	1.34	1.50
TWMR [kcal/min]	4.51 ± 0.536 (3.45-5.95)	3.76	4.07	4.47	4.83	5.47
BMR / BM [cal/min/kg]	18.4 ± 1.88 (14.7-23.4)	15.4	17.2	18.2	19.9	21.6
TWMR / BM [cal/min/kg]	66.1 ± 5.05 (58.3-79.1)	58.9	62.0	65.9	69.7	74.9
BMR / FFM [cal/min/kg]	20.6 ± 2.09 (16.8-26.0)	17.3	19.3	20.2	22.3	24.9
TWMR / FFM [cal/min/kg]	74.2 ± 6.68 (63.5-89.1)	64.6	69.3	73.6	78.8	87.9
TWMR / BMR	3.61 ± 0.329 (2.93-4.47)	3.13	3.36	3.58	3.87	4.17
$BMR/BM^{.68}$ [cal/min/kg)	69.43 ± 6.39 (57.5-84.5)	58.8	65.4	68.6	73.7	81.4
$TWMR/BM^{.81}$ [cal/min/kg]	147.4 ± 10.56 (127.5-174.6)	133	139	146	154	164

Table 2.11.B. Distribution of the data of the subjects that performed the treadmill walking metabolic rate study. Males (n = 76).

BM = body mass; FFM derived from UWW.
cal = calories = 0.001 kcal

3.2. Prediction of TWMR.

TWMR prediction from simple ratio standard.

a) Simple ratio standard (SRS) between TWMR with BMR. The SRS refers to the same term as the multiplicative factor. FAO/WHO/UNU expresses physical activities as multiples of BMR, i.e. walking on the level at normal pace, for females (BMR*3.4) and for males (BMR*3.2) kcal/min. On this basis it was deemed appropriate to find what the multiplicative factor of BMR would be for the standardized walking of this study, i.e., on the level at 4.8 km/h.

When TWMR was divided by each subject's BMR the following data was obtained:

TWMR/BMR	mean ± SD [kcal/min]	range	C of V [%]
Females	3.17 ± 0.262	2.58 to 3.95	8.26
Males	3.62 ± 0.329	2.93 to 4.47	9.09

Table 2.12. Multiplicative factors of BMR for the standardized walking metabolic rate.

Variation between subjects is wide. However, for females no variable could explain variation any further in this ratio. For males, B.M. explained a further 10% (r=0.25; p=0.04) and age a further 6% (r = 0.33 p = 0.004).

The ANOVA of TWMR/BMR showed the following equations:

TWMR [kcal/min] =

Females (n=76): 3.17 * BMR (± 0.049); RSD = 0.262

Males (n=73): 3.62 * BMR (± 0.035); RSD = 0.329

For both sexes (n = 149) RSD = 0.297

These ratio values: 3.2 for females and 3.6 kcal/min for males, are somehow different, specially for males, to the ones of FAO/WHO/UNU: 3.4 and 3.2 kcal/min, respectively. It is note-worthy that the multiplicative factor of FAO/WHO/UNU is a bit greater for females than for males and that it is not specified the speed of "walking at normal pace".

b) The SRS between TWMR and B.M. is:

TWMR [kcal/min] =

Females: 0.053 * B.M. (± 0.0006) RSD = 0.259

Males: 0.066 * B.M. (± 0.0005) RSD = 0.343

c) The SRS between TWMR and FFM is:

TWMR [kcal/min] =

Females: 0.066 * FFM (± 0.0009) RSD = 0.279

Males: 0.074 * FFM (± 0.0007) RSD = 0.405

TWMR from linear Regression Analysis.

a) by linear regression analysis TWMR on BMR, the following equations were obtained:

TWMR [kcal/min] =

Females: 0.53 (± 0.220) + 2.59 * BMR (± 0.234); RSD = 0.232

Males: 1.33 (± 0.380) + 2.54 * BMR (± 0.302); RSD = 0.382

By ANOVA it was found that the constant term was significantly different between sexes, but the multiplicative factor of BMR (or slope) was not so, the obtained equation is:

TWMR [kcal/min] =

Females: 0.56 (± 0.185) + 2.56 * BMR (± 0.195); RSD = 0.23

Males: 1.31 (± 0.247) + 2.56 * BMR (± 0.195); RSD = 0.38

RSD for both sexes (n = 149): 0.313; r^2 = 0.883

b) The simple linear regression of TWMR on B.M. showed the following results:

TWMR [kcal/min] =

Females: 0.548 (±0.253) + 0.043*BM (±0.005) r^2= 0.551; RSD = 0.253

Males: 0.961 (±0.321) + 0.052*BM (± 0.005) r^2= 0.637; RSD = 0.325

The ANOVA analysis showed either the constant term (p = 0.31) or the slope (p = 0.20) to be non-significantly different between sexes. If the constant term was taken as the non- significant term then, r^2 = 0.9;

TWMR [kcal/min] =

Females: 0.758 (±0.204) + 0.039 * BM (±0.0037) RSD = 0.252

Males: 0.758 (±0.204) + 0.055 * BM (±0.0030) RSD = 0.324

If the constant term was taken as the non-significant term then, r^2 = 0.8991;

TWMR [kcal/min] =

Females: 0.254 (±0.064) + 0.0485* BM (±0.0033) RSD = 0.253

Males: 1.191 (±0.226) + 0.0485* BM (±0.0033) RSD = 0.324

The three equations are very similar in terms of the RSD which changes insignificantly. It is difficult to select the best choice, but it would seem that the equations with same constant and different

slope are better, the reason for this is because both the constant term and the slope are closer to both the constant term and the slope when each sex is regressed separately. The third option, in which the slope is the same for both sexes, the constant term changes a lot from the first option.

c) The linear regression of TWMR on FFM showed the following results:

TWMR [kcal/min] =

Females: 0.599 (±0.285) + 0.053*FFM (±0.006) r^2= 0.485; RSD = 0.272

Males: 1.322 (±0.368) + 0.052*FFM (±0.006) r^2= 0.518; RSD = 0.375

The ANOVA analysis showed the slope to be non-significantly different between sexes (p=0.96). The following equations were obtained (r^2 = 0.87);

TWMR [kcal/min] =

Females: 0.612 (±0.089) + 0.052*FFM (±0.0043) RSD = 0.252

Males: 1.314 (±0.265) + 0.052*FFM (±0.0043) RSD = 0.324

TWMR from power function ratio standard.

The log TWMR was regressed on log-BMR, log-B.M. and log-FFM to find the appropriate power function ratio standard (PFRSt). In the three instances the PFRSt were not statistically significant different between sexes and the ANOVA showed the multiplicative factor to differ between sexes.

a) For log-BMR, the PFRSt was of 0.768; the following equations were obtained (r^2 = 0.9934; RSD = 0.3133 kcal/min):

TWMR [kcal/min] =

Females: 3.11 (± 0.038) * $BMR^{.77}$ RSD = 0.231

Males: 3.80 (± 0.031) * $BMR^{.77}$ RSD = 0.381

b) For the log B.M. the PFRSt was found to be = 0.81. The obtained equations were (r^2 = 0.994):

TWMR [kcal/min] =

Females: 0.114 * $BM^{.81}$ (± 0.001). RSD = 0.251

Males: 0.147 * $BM^{.81}$ (± 0.001). RSD = 0.324

c) For the log-FFM the PFRSt was: 0.76. The equations obtained were as follows (r^2 = 0.993):

TWMR [kcal/min] =

Females: 0.165 * $FFM^{.76}$ (± 0.002). RSD = 0.270

Males: 0.198 * $FFM^{.76}$ (± 0.002). RSD = 0.374

3.3. Selection of the best single TWMR predictor.

The error of prediction (RSD) is a way to select among the above models (simple ratio standard, linear regression or power function ratio standard) and variables (BMR, BM or FFM) the best single TWMR predictor:

Model	Females	Males	Both
BMR			
Simple Ratio	0.262	0.329	0.297
Linear Regression	0.230	0.379	0.313
Power Function	0.231	0.381	0.313
Body Mass			
Simple Ratio	0.259	0.343	0.303
Linear Regression	0.252	0.324	0.291
Power Function	0.251	0.324	0.289
F F M			
Simple Ratio	0.279	0.405	0.347
Linear Regression	0.252	0.324	0.326
Power Function	0.270	0.374	0.325

Table 2.13. Errors of prediction of TWMR from BMR, body mass and FFM by simple ratio standard, linear regression and power function ratio standard.

When TWMR was predicted from BMR it was found that for females the SRS model gave the greatest prediction error and for males and for both sexes, the smallest. This model is the same as the multiplicative factor of BMR. The other two models gave practically the same error.

When TWMR was predicted from either from B.M. or from FFM, it was found that either the LRA model or the PFRSt model gave the same prediction errors and the SRS model gave the greatest errors. Prediction errors were smallest for males, when B.M. was the predictor variable rather than BMR or FFM and for females, when BMR by either LRA or the PFRS models.

Best TWMR predictors among BMR, body mass and FFM variables. A stepwise multiple regression analysis was performed on each sex to find out among the variables: BMR, BM, FFM or its combination, explained more of BMR variation.

For females (n=76), the first variable selected was BMR explaining 62.3% of TWMR variance with a RSD = 0.232 kcal/min; then, $BM^{-.81}$ was selected increasing the prediction to 69.6% with a RSD = 0.21, and the constant term became not significant (p=0.56). Leaving out the constant term BMR and $BM^{-.81}$ explained 99.5% of TWMR and the RSD = 0.21 kcal/min. The equation obtained is:

$$TWMR\ [kcal/min] = 1.71 * BMR\ (\pm 0.29) + 0.052 * BM^{-.81}\ (\pm 0.01)$$

For males (n=73), B.M. was the first selected variable explaining 63.7% of TWMR variance with a RSD = 0.325 kcal/min and then BMR which increased the prediction to 71.3% with a RSD = 0.29 kcal/min, and the constant term became n.s.; leaving this term out, the variables selected changed to $BM^{-.81}$ and BMR, explaining 99.6% of TWMR with a RSD = 0.29 kcal/min. The equation obtained is:

$$TWMR\ [kcal/min] = 1.204 * BMR(\pm 0.28) + 0.098 * BM^{-.81}\ (\pm 0.01)$$

An ANOVA was performed to study whether the slopes of both variables, BMR and $BM^{-.81}$, were statistically different between sexes. It was found that the multiplicative factor of BMR was not significantly different between sexes (p=0.29), but that of $BM^{-.81}$ was different (p = 0.03). The obtained equations were:

TWMR [kcal/min] =

Females: $1.37 * BMR\ (\pm 0.20) + 0.065 * BM^{-.81}\ (\pm 0.007)$

Males: $1.37 * BMR\ (\pm 0.20) + 0.092 * BM^{-.81}\ (\pm 0.008)$

Best TWMR predictors among all measured body composition variables. A stepwise multiple regression analysis was performed on each sex to predict TWMR by BMR, BM, $BM^{-.81}$, FFM, age, arm, calf, tight and buttocks circumferences and the Σ3circ., bone diameters and its sum.

The best variables chosen for females, (n=75) were BMR, buttocks (But. Circ.) and calf circumferences (calf Circ.) and for males, (n=73) there were B.M. and then BMR. The following order of equations was got:

TWMR [kcal/min] =

Females (n=75):

1) $0.54\ (\pm 0.23) + 2.58 * BMR\ (\pm 0.238)$; $r^2 = 0.62$; RSD = 0.234

2) -1.47 (± 0.52) + 0.029 * But.Circ. (± 0.007) + 1.90 * BMR (± 0.27); r^2 = 0.69; RSD = 0.211
3) -1.82 (± 0.54) + 0.022*But.Circ. (± 0.007) + 1.73*BMR (± 0.28) + 0.032 * Calf Circ. (± 0.015); r^2 = 0.71; RSD = 0.206

Males (n=73):
1) 0.96 (± 0.32) + 0.052 * BM (± 0.0046); r^2 = 0.64; RSD = 0.325
2) 0.343 (± 0.32) + 0.038 * BM (± 0.005) + 1.25 * BMR (± 0.29) r^2 = 0.71; RSD = 0.291

3.4. Relationship of TBK on Treadmill Walking Metabolic Rate.

Total body potassium was studied separately to know the effect of a FFM component, i.e., muscle mass, on TWMR.

The regression analysis of TWMR on TBK showed r^2 values of 0.44 for females and 0.28 for males. An ANOVA showed the slopes for TBK to be non significantly different between sexes but the constant term was different; an allowance was made for this and the obtained equation reached an r^2 = 0.840 and a RSD = 0.364.

TWMR [kcal/min] =
Females: 1.437 (±0.151) + 0.00052*TBK [mmol] (±0.0001) RSD = 0.231
Males: 2.372 (±0.411) + 0.00052*TBK [mmol] (±0.0001) RSD = 0.432

When either BMR, B.M. or FFM were added, TBK became non significant. Then, TBK added nothing to the prediction of TWMR.

When stepwise multiple regression analysis of TWMR was performed on all B.C. variables including TBK, the number of females was = 26 and of males = 38. For either sex, TBK was not included among the variables that best explained TWMR.

For females, $BMR^{.77}$ (r^2 = 0.66; RSD = 0.184) and the Σ3circ (r^2 = 0.75; RSD = 0.163) were selected.

For males B.M. (r^2 = 0.55; RSD = 0.348), BMR (r^2 = 0.64; RSD = 0.312) and $FFM^{.76}$ (r^2 = 0.713; RSD = 0.284) were selected.

3.5. Relationship between TWMR/BMR, TWMR/BM and TWMR/FFM with body mass, FFM, and TBK.

Relationship	Females (n=76) r = ; p =	Males (n=76) r = ; p =
TWMR/BMR on BM	0.10; n.s.	0.25; 0.04
TWMR/BMR on FFM	-0.04; n.s.	0.12; n.s.
TWMR/BM on BM	-0.24; 0.04	-0.34; 0.003
TWMR/FFM on FFM	-0.23; 0.04	-0.39; 0.0006
TWMR/FFM on BM	-0.03: n.s.	-0.20; n.s.
TWMR/BM on TBK	(n=27) 0.13; n.s.	(n=38) -0.46; 0.003
TWMR/FFM on TBK	(n=27) -0.15; n.s.	(n=38) -0.52;0.0008

Table 2.14. Relationship between TWMR/BMR, TWMR/BM and TWMR/FFM with B.M., FFM, and TBK.

TWMR/BMR showed a trend to increase from light to heavy males (because BMR on B.M. is significant).

TWMR/BM decreased from light to heavy individuals of both sexes.

TWMR/FFM did not show any significant trend as B.M. increased for either sex.

However, TWMR/FFM significantly decreased as FFM increased, which probably reflects a higher muscle mass. This was proved using TBK as indicator of muscle mass, in those subjects that had it measured.

In those males that had TBK measured (n=38), TWMR/FFM on FFM and TWMR/BM on B.M. significantly decreased (r=-0.52; p=0.0008 and r=-0.44; p=0.006, respectively) and also did both relations TWMR/BM and TWMR/FFM on TBK (r=-0.51; p=0.0008 and r=-0.51; p=0.003, respectively).

In those females that had their TBK measured (n=26), the relation TWMR/FFM on FFM was non significant (r=-0.27; p=0.17) and TWMR/BM on B.M. was weak but significant (r=-0.21; p=0.04). The relations TWMR/BM and TWMR/FFM on TBK were both non significant (r=-0.15 and 0.13, respectively).

Then, the decrease of TWMR per kg of B.M. or FFM as mass increased could be explained by a greater amount of muscle mass in those males that had their TBK measured. In those females that had

their TBK measured there was not a significant decrease of TWMR/FFM as mass increased; then, it was obvious that TBK had nothing to explain.

4. GENERAL SUMMARY.

This study sought to analyse the relationship between BMR and B.M., FFM and other B.C. variables related to muscularity, fatness and body frame, age and sex in 157 lean-muscular individuals and to evaluate the extent to which walking metabolic rate (WMR), as example of an important component of E.E., may best be expressed as multiple of BMR or per kg of B.M. or FFM.

B.M. and FFM prediction of BMR were evaluated. Similarly, B.M., FFM and BMR prediction of treadmill WMR (TWMR) were evaluated. Three different mathematical models were compared: simple ratio standard (SRS), power function ratio standard (PFRS) and linear regression analysis (LRA). All other B.C. variables that indirectly indicate muscularity, fatness and body frame were evaluated.

The C.V. within sexes in BMR was found to be of ≈ 12% for both sexes; SD for females = 166 kcal/day; SD for males = 216 kcal/day. BMR variance was best explained by differences in FFM. The ANOVA showed that the slope was not significantly different between sexes but the constant term was. Still, the RSD was 102 kcal/day for females and 148 kcal/day for males.

Comparison among the three mathematical models showed the LRA to give the least RSD, but very similar to the PFRS model.

At a given value of FFM or B.M. ($\pm$ 2.5 kg) C.V. between 6 to 13% were found. Therefore, for the purpose of predicting an individual's BMR, FFM was found to be no better than B.M..

While the use of FFM almost eliminated differences in BMR between sexes, between methods' variation in the estimation of FFM were important.

A fall of the BMR per kg of FFM from light to heavy individuals was found and the use of the PFRS overcame this fall. Differences in the composition of FFM between subjects have been suggested as the physiological reason, i.e., heavier individuals are more muscular and their BMR/FFM decreases because of the low metabolic rate of muscle. An important finding of this study was that TBK, as an indirect index of muscularity, explained this decrease, although only in males.

In females (n=28) the inclusion of TBK improved in 10% the BMR prediction by B.M. and in 2% the prediction by FFM. In males (n=38) inclusion of TBK improved in 5% the BMR prediction by B.M., but when age was included TBK was not further significant. As the only

predictor variable TBK explained more of BMR variation than B.M. in both sexes (females 52%, RSD = 100 kcal/day; males 36%, RSD = 161.4 kcal/day).

When all B.C. variables were included to perform a stepwise multiple regression analysis, for females, FFM was chosen in the first place followed by arm muscle area and the sum of three circumferences: arm, thigh and calf, with a RSD = 105 kcal/day, but the improvement in BMR by the last two circumferences was of only 8% above FFM (RSD = 113 kcal/day). Alternatively, B.M. and calf circumference gave a similar prediction. For males who had TBK measured (n=38), the sum of 4 diameters, age and TBK were first selected (RSD = 148 kcal/day) and, for all males (n=78) the sum of 4 diameters, age and B.M. (RSD = 152 kcal/day) were preferred.

As it is customary, in practice, to use predictive equations to estimate BMR, a comparison was done between measured BMR and BMR calculated by some of the most popular predictive equations. This comparison showed that the current FAO/WHO/UNU equations underestimated, though not significantly, the measured value in females (mean difference = 1.7 $\pm$ 9.58) kcal/day and significantly underestimated it in males (5.6 $\pm$ 9.41). In males, the Dubois & Dubois and Cunninhams' estimations did not significantly differ from the measured BMR. Comparisons with other predictive equations are also presented.

The TWMR was measured at a constant pace of 4.8 km/h. Using the error of prediction it was possible to evaluate the variable (B.M., FFM or BMR) and model that more accurately estimated WMR.

The multiplicative factor of BMR for walking was 3.2 for females and 3.6 for males; these values are slightly different from those of FAO/WHO/UNU, 1985.

The variable that best explained the TWMR variance in females was BMR by either LRA (r^2 = 0.62) or PFRS models (RSD = 0.23 kcal/min) and, for males, either B.M. by the LRA (r^2 = 0.64) or PFRS or FFM by LRA model (RSD = 0.32 kcal/min). It was interesting to find that estimating TWMR as a multiple of BMR as FAO/WHO/UNU recommends was not the most appropriate way to do it.

By the stepwise multiple regression analysis it was possible to decrease a bit further the RSD when all measured B.C. variables were included; for females, the variables chosen were BMR, buttocks and calf circumference (r^2=0.71; RSD=0.21) and for males B.M. or $BM^{.85}$ and BMR (r^2=0.71; RSD=0.29). TBK added nothing to the prediction of TWMR but, as indirect indicator of muscularity, it was able to explain part of the decrease of TWMR per kg FFM from light to heavy males.

CHAPTER 3

BODY COMPOSITION

LITERATURE REVIEW

1. CHEMICAL AND ANATOMICAL ANALYSES IN CADAVERS.

Information on human body composition (B.C.) from cadaver analysis is scarce, probably because of the difficulty in obtaining normal healthy bodies. The integration of the data of the B.C. of cadavers with different physical conditions and characteristics, analysed by different authors using various techniques, make the variation of the data to seem wider than it might actually be; even though the best and most objective data on B.C. is derived from the analysis of human cadavers, it must be taken with some caution. Even careful analysis of cadavers encounters severe technical difficulties because of the quantity of the material that must be handled.

Womersley, 1974 compiled the information on 8 adult human cadavers, 6 males and 2 females, which had been subjected to careful chemical analysis (figure 3.1.), four males analysed by Mitchell and his co-workers in the United States (Mitchell et al, 1945; Forbes et al, 1953, 1956), 2 males and a female by Widdowson et al, 1951 and a female by Moore et al, 1968 in Great Britain. Seven were Caucasians and one Negro (Forbes et al, 1956). Womersley found that none of these cadavers appeared completely normal at post mortem examination; some of these cadavers were edematous and so, based on the variability of the water content of the FFM for animals of different species (70-78%), he assumed that the range of values for humans should be narrower and as the variability found for the cadavers was 69-82% he used the value of 72.5% to correct for the water content of the FFM of 3 cadavers.

If the above mentioned cadavers were actually edematous, it would be difficult to accurately know the amount of water retained and, if this is the case the body mass (B.M.) and FFM would be overestimated. The correction performed by Womersley would have to had taken this into account but he used the original B.M. and FFM reported. If, instead of correcting the data of those cadavers suspicious of presenting edema, they were eliminated, the overall mean data of the components of the FFM of the 4 more reliable cadavers (two males analysed by Forbes et al, 1953 & 1956; and a male and a female analysed by Widdowson et al, 1951) becomes:

water [%] 71.3 $\pm$ 1.8 (69.4-73.2);
protein [%] 21.5 $\pm$ 2.5 (19.2-23.8);
mineral [%] 7.2 $\pm$ .43 (6.8-7.6);
potassium [mmol/kg FFM] 69.3 $\pm$ 3.2 (66.5-72.6);
calcium [g/kg] (21.3-24.8).

BODY COMPOSITION	FEMALES (n = 2)	MALES (n = 6)	OVERALL (n = 8)
AGE [years]	55 [42-67]	44 ± 12.1 [25-60]	46.4 ± 13.2 [25-67]
BODY MASS [kg]	44.3 [45.1-43.4]	65.9 ± 7.5 [53.8-73.5]	60.5 ± 11.9 [43.4-73.5]
HEIGHT [cm]	169	174.4 ± 6.3 [169-183]	173.5 ± 6.1 [169-183]
FAT [%]	16.2 [8.8-23.6]	13.4 ± 9.8 [1.1-27.9]	14.1 ± 9.3 [1.1-27.9]
FFM [kg]	37.1 [34.5-39.6]	57.0 ± 7.5 [43.3-63.1]	51.9 ± 11.3 [34.5-63.1]
FFM COMPOSITION			72.0 ± 1.4 [69.4-73.2]
Water %	72.9 [72.5-73.2]	71.7 ± 1.5 [69.4-73.0]	
Protein %	19.6 [19.2-20]	21.3 ± 1.8 [19.5-23.8]	20.9 ± 1.7 [19.5-23.8]
Mineral %	7.6 [7.5-7.6]	6.9 ± 0.6 [6.0-7.6]	7.1 ± 0.6 [6.0-7.6]
K [mmol/kg]	56.4 [40.2-72.6]	64.5 ± 7.6 [53.7-71.4]	61.8 ± 12.5 [40.2-72.6]
Ca [g/kg]	23.8 [22.7-24.8]	22.6 ± 1.7 [20.6-25.1]	22.9 ± 1.7 [20.6-25.1]

Figure 3.1. Some general characteristics and body composition (mean ± S.D. and ranges) of 8 human cadavers subjected to chemical analysis.

The coefficients of variation (CV) of the components of the FFM also change. i.e., water CV increases from 1.9 to 2.6% and protein's from 8.1 to 11.5%; mineral CV decreases from 8.5 to 6.1% and the most important shift is a decrease on the CV of the amount of potassium of the FFM from 20.2 to only 4.6%. As it can be seen, the exclusion of the cadavers with doubtful data would not significantly change the mean values nor its variation, except for potassium.

Even though there are variations, it can be seen that the relative proportions of the components of the FFM are more or less fixed. However, as Siri, 1956 states, these few cadavers could hardly provide average values for normal humans for obvious statistical reasons and even more because of the circumstances leading to death.

More data is available on human cadavers subjected to anatomical dissection, there are 42 adult cadavers whose major tissue masses have been weighed. For the five cadavers chemically analysed by Mitchell & col. (Mitchell et al, 1945; Forbes et al, 1953, 1956), and by Moore et al, 1968 anatomical composition is also available and, besides, there is data of another 12 human cadavers of the 19th century, compiled by Womersley, 1974 and by Clarys and Martin, 1985. Most of these subjects had died suddenly and were previously in good health.

Another, more recent, source of information on cadavers is "The Brussels Cadaver Study" (B.C.S.), which was carried on by Clarys, Martin and Drinkwater in 1984. They dissected and analysed 25 Belgian human cadavers (13 females and 12 males) of elderly subjects (mean age, 76 years) reasonable intact and non-emaciated.

Anatomical dissection included the data of adipose tissue and adipose tissue free mass (ATFM) or lean body mass; although these compartments are not the same as fat and FFM, the compartments included in the chemical analysis, the change in composition in one will be reflected in the other. Anatomical dissection includes in the ATFM compartment all the organs, tissues and fluids in the body but adipose tissue, namely: skeletal muscle, skeleton, viscera, skin, blood and nerve. The mean chemical composition of the adipose tissue of 4 cadavers analysed by Mitchel et al, 1945 and by Forbes et al, 1953 & 1956, is: fat 49% (4.2-78.4, CV 69%); protein 8% (5.9-12.8, CV 38.5%) and water 43% (16.8-83.9, CV 70%). The variation depends, on the authors belief, on the degree of fatness.

Figure 3.2. shows the mean data of these cadavers:

- The first value in the table refers to the mean of the 19th century cadavers. For females, because there are only 2, the actual values

are presented and not the mean value; for males the mean value of 10 cadavers is presented ± 1 S.D. and range in brackets. The overall mean includes the data of the 12 cadavers.

- The second value is the mean value of the 4 male cadavers analysed by Mitchell et al, 1945 and Forbes et al, 1953 & 1956, (Mit & Forbes). the data of Moore et al, 1968 was not included because of the edema of this cadaver.

- The third value is the mean value of the Brussels cadavers study (B.C.S.) there were 13 females and 12 males.

- The overall mean includes data of the 25 cadavers.

As it can be seen from figure 3.2.B., there is a considerable variation in the composition of the components of the adipose tissue free mass (ATFM). The coefficients of variation (CV) for muscle and for skeleton for the overall group showed values of 10 and 11%, respectively. If the Brussels Cadaver Study (B.C.S.) were not included, then the CV for the other 16 cadavers is 11 and 8.6%, respectively. The B.C.S. presented CV of 8.8% for muscle and 12.6% for skeleton. As it can be seen muscle is more variable for the first 16 cadavers and skeleton is more variable for the 25 cadavers analysed by Clarys & col. (B.C.S.). The variation in muscle might be explained by the amount of adipose tissue that is possible to dissect from the tissues, this should be difficult to standardize even within one group of workers; then, if the analyses are performed by different groups of researchers, the possibility of variation would obviously be higher. The variation of the skeleton could be due more to physiological than to methodological facts; the cadavers from the B.C.S. were on average, older than the other 16 cadavers. Besides the normal inter-subject variation, it is known that a decrease in the amount of mineral of the bones happens with aging, specially in women just after the menopause. In the B.C.S., women presented a higher CV (16.8%) in the proportion of skeleton of the ATFM and the whole group of 25 cadavers, a CV of 12.6%; it could had been that some of the subjects had already lost or were losing mineral at the time they died while others had probably their normal mineral content, probably because they were younger or practiced some physical activity that prevented them for losing it.

BODY COMPOSITION	STUDY	FEMALES	MALES	OVERALL
AGE [years]	19th.Century	22, 55	36 ± 9.7 [26-50]	36 ± 12.1
	Mit.& Forbes	-	47 ± 10.2 [35-60]	
	B.C.S.	76	76	76
	Overall			53
BODY MASS [kg]	19th.Century	55.4, 46.0	59.3 ± 8.7 [52.7-76.5]	57.9 ± 8.8
	Mit.& Forbes	-	65.0 ± 8.9 [53.8-73.5]	65.0 ± 8.9
	B.C.S.	62.5 ± 9.4 [48.2-75.4]	66.2 ± 12.5 [51.7-88.9]	64.3 ± 10.9
	Overall			62.9 ± 10.4
HEIGHT [cm]	19th.Century	159, 160	169 ± 8.3 [157-184]	166.5 ± 8.2
	Mit.& Forbes	-	173 ± 6.7 [169-183]	173 ± 6.7
	B.C.S.	-	-	-
	Overall			-
ADIPOSE TISSUE [kg]	19th.Century	15.7, 8.1	6.9 ± 3.6 [1.0-12.6]	7.8 ± 4.1
	Mit.& Forbes	-	8.7 ± 5.4 [3.2-15.9]	8.7 ± 5.4
	B.C.S.	25.8 ± 7.8 [14.4-40.1]	20.0 ± 8.4 [9.7-43.3]	23.0 ± 8.5
	Overall			17.1 ± 10.2
ADIPOSE TISSUE FREE MASS [kg]	19th.Century	39.7, 37.9	52.5 ± 5.7 [45.9-65.5]	50.2 ± 7.4
	Mit.& Forbes	-	56.3 ± 5.9 [47.7-61.0]	56.3 ± 5.9
	B.C.S.	36.7 ± 4.4 [29.6-44.9]	47.7 ± 10.4 [32.4-68.1]	42.0 ± 9.5
	Overall			45.8 ± 9.8

Figure 3.2.A. Data (mean ± S.D. and ranges) of 41 human cadavers subjected to anatomical dissection.

BODY COMPOSITION	STUDY	FEMALES	MALES	OVERALL
Skeletal muscle [%]	19thCentury	50.0, 41.2	47.7 ± 5.1 [33.9-51.0]	47.4 ± 5.1
	Mit&Forbes	-	44.5 ± 6.1 [36.6-51.4]	44.5 ± 6.1
	B.C.S.	48.1 ± 3.8 [41.9-54.8]	52.0 ± 4.3 [45.3-59.4]	50.0 ± 4.4
	Overall			48.7 ± 4.9
Skeleton [%]	19thCentury	21.1, 23.0	20.8 ± 1.3 [19.4-23.1]	21.0 ± 1.4
	Mit&Forbes	-	18.4 ± 1.3 [17.0-19.9]	18.4 ± 1.3
	B.C.S.	21.3 ± 1.8 [17.4-25.7]	19.9 ± 2.4 [16.3-24.8]	20.6 ± 2.3
	Overall			20.5 ± 2.3
Skin [%]	19thCentury	8.0, 7.4	6.8 ± 0.8 [5.6-8.5]	7.0 ± 0.8
	Mit&Forbes	-	8.7 ± 1.2 [7.2-10.0]	8.7 ± 1.2
	B.C.S.	9.2 ± 1.1 [8.0-11.4]	7.8 ± 0.8 [6.1-9.1]	8.5 ± 1.2
	Overall			8.1 ± 1.3
Residual [%]	19thCentury	20.9, 28.5	24.6 ± 5.7 [21.1-40.3]	24.6 ± 5.4
	Mit&Forbes	-	28.4 ± 6.8 [21.0-37.4]	28.4 ± 6.8
	B.C.S.	21.3 ± 1.8 [18.7-24.0]	20.4 ± 2.6 [16.3-24.6]	20.9 ± 2.3
	Overall			22.7 ± 4.5

Figure 3.2.B. Proportion of tissue masses of adipose tissue free mass of 41 cadavers subjected to anatomical dissection.

It is evident from the comparison of this figure with figure 3.1., that the data of anatomical dissection of cadavers is more variable than the data of chemical analysis. The reason is that chemical analyses are better standardized, while the manual separation of all dissectable adipose tissue, the removal of blood and other components from each organ or tissue must, of course, cause more error. It is a shame that the physical condition of those cadavers exposed to chemical analysis were not in good conditions whereas most of the cadavers that were anatomically dissected were in a more healthy state. As the error associated with anatomical dissection is greater than that of chemical analysis, the true variability between subjects must be smaller than the one presented herein, specially if the cadavers are from the same race, sex and of similar age and physical conditions.

2. IMPORTANCE OF ASSESSING THE COMPOSITION OF THE BODY (B.C.).

The amount of fat may influence morbidity and mortality; it alters the efficacy of drugs and anaesthetic action, influences the tolerance to cold and starvation and affects metabolic rate. The knowledge of B.C. is necessary for the assessment of nutritional status and the prognosis of and the recovery from illness.

In the relation of health to fatness, overweight individuals have been found to have a higher incidence of certain diseases (specially cardiovascular) and a higher mortality rate which increase with the proportion of overweight. However, it is not certain in which cases death is caused by an excess of fat or whether it is associated with other factors.

For the treatment of malnutrition, either obesity or undernutrition, it is necessary to know the degree of severity, for the correct planning of weight loss or gain.

For the performance of certain sports it is convenient to know the physical configuration of the subject so that the subject may chose the sport which may augur excel or else to try and induce some changes when possible.

For the effect of the type and intensity of physical activity on B.C.: fatness and muscular development.

For the assessment of physical work capacity, the maximum rate of oxygen consumption is intimately related to the FFM. Von Döbeln, 1956 has proposed that resting oxygen consumption is directly related to the $FFM^{0.64}$.

Variability of basal metabolic rate (BMR) between individuals has been tried to be explained on the basis of B.C. Lewis, 1991 found in a group of 97 healthy women, that differences in BMR between them were explained by differences in FFM, accounting to 45% of the total

variance; however, at a given FFM considerable variation in the BMR was still evident.

For the assessment of growth and aging in the community, to know whether growth and changes with aging are occurring within desirable limits.

For the establishment of energy requirements in pregnancy; Durnin & col. 1982-1991 have studied this matter and since about half of the total extra energy needed is to lay down about 3-4 kg of adipose tissue to provide an extra store of energy for the needs of late pregnancy and of lactation, its exact measurement is an important part of the investigation.

Some relations have also been found between personality and behaviour with B.C. Tanner, 1964 found that certain careers tend to be chosen by people of a specific physical type.

For description of different groups of sex, age and ethnic groups.

3. GENERAL DESCRIPTION OF THE DIFFERENT TYPES OF TECHNIQUES TO MEASURE BODY COMPOSITION.

Many methods for measuring body composition (B.C.) have become available from the 1940's, since the work of Behnke and others; most of them are based on the common assumption that the body can be divided into two compartments which composition is essentially constant between individuals; although, as it has just be seen, this is not entirely true but, it can be fairly assumed to be so. During the 1960's the progress of the study on B.C. and the application of knowledge went fast and then it stabilized and it appeared that continuing work in this area would merely involve the application of existing techniques. However, in the 1980's more techniques have become available making possible the study of newer aspects of B.C. and health but the simple separation of the body into two compartments have been made more complex.

There are some techniques which may only be practiced at laboratory level, are expensive and applicable only to few subjects, and at the other extreme there are those which are applicable to field and population studies, that are fast and cheap, such as the application of previously derived equations using anthropometric variables, but might not be as accurate.

Most of the applications mentioned in section 2. require that measurements are made on a large number of people; therefore many of the techniques that need expensive or specialized equipment, will be inappropriate for these purposes; rather those techniques which require measurements that are easy, fast, cheap, non-invasive,

accurately enough and possible to be performed in field conditions shall be the ones to be more useful.

4. THE TWO COMPONENT MODEL OF BODY COMPOSITION.

For the study of its composition the body can be divided in several forms. One of the most common and useful is the classification into two compartments (Keys and Brozek, 1953). A chemical and anatomical distinction should be mentioned because this has led to much confusion in the used terminology. Fat and fat free mass (FFM) refer to the chemical composition while adipose tissue and adipose tissue free mass (ATFM), or lean body mass (LBM), is the anatomical analogue. It is often found in the literature that these terms are used interchangeably but they are not the same.

Fat Mass: this compartment includes the entire content of chemical fat or lipids in the body; i.e., the fat stored in subcutaneous, intra-thoracic, intra-abdominal, and the essential lipids included in the membranes, the central nervous system and bone marrow. It is anhydrous, contains no potassium, has a fairly constant density of about 0.9 g/cm^3 and it is defined as the ether-extractable constituent of the body.

Fat free mass (FFM): includes the mass of all tissues and fluids in the body but fat; chemically it comprises: water, protein and mineral. The composition of this compartment is assumed to be constant, i.e., a water content of about 72%, mineral of about 7% and protein of about 21%; its potassium content is about 68 mmol/kg and its density of about 1.100 g/cm^3.

Adipose tissue: is made up of fat, protein or 'cell residue' and water in a variable proportions which depends on the fatness of the individuals. Adipose tissue may contain 10 - 30 % water, but the exact proportion is not known nor is it certain that the proportion is constant for various degrees of obesity (Siri, 1957). Brozek et al, 1963 have proposed the following mean estimations: 64% fat; 22% water and 14% protein and Garrow, 1982: 83 % fat; 15 % water and 2% protein.

Lean body mass (LBM), or adipose tissue free mass (ATFM): includes the mass of all dissectable tissues and fluids less adipose tissue. Essential lipids, not contained in adipose tissue are included in this compartment. Anatomical components are those which may be isolated by dissection and it includes the visceral organs, skin, skeletal muscle, skeleton, nervous tissue, blood and connective tissue.

Under the assumption that the body consists of 2 chemically distinct compartments of relatively constant composition, the

estimation of its composition can be assessed by measuring the density, water or potassium content of the body to allow a calculation to be made of the composition of the body by difference with the whole body mass.

The development of new technology has made possible the approach of measuring the four body's main functional constituents: water, protein, mineral and fat, obtaining a direct measurement of a particular element or compound. Using a model of these four chemical groups, multi-compartment measurements are being performed. However, many of these 'new methods' may not be all together available or non-accessible for cost and not convenient for field studies and all this much information may not be needed. Even more, as Siri, 1956 stated, in a direct method, such as that of extracellular fluid, the space that is observed depends upon how it is measured and, while it may be said that the most reliable methods give a reasonable value for extracellular fluid, the error is still comparable to the magnitude of changes and differences that are of most interest.

The selection of the correct method to estimate B.C. will depend on the objectives and resources of the study; however it must be stressed that the main limitation for its accurate estimation is the biological variability of the composition and density of the FFM in different individuals with varying sex, age, ethnicity, health status and physical activity.

The present research deals with the analysis of the basic assumptions used by some techniques based on the scheme of two compartments of constant composition on healthy subjects. Following is a description of those methods that use the scheme of a two compartment model and that will be used in this thesis.

5. DENSITOMETRY.

The density of the body can be estimated using hydrostatic weighing which is one of the first and most widely used methods to estimate body composition (B.C.).

It employs the Archimede's Principle, which states that the volume of an object submerged in water equals the volume of water it displaces. Probably the first workers to measure body density (B.D.) for the purpose of estimating its fat content were Behnke et al, 1942.

The basic assumption made when estimating body fat by densitometry (D) is that the body consists of two compartments, fat mass and fat free mass (FFM), which have distinctly different and constant densities. At 37°C, FFM has been estimated to have an

average density of about 1.100 g/cm^3 (Behnke et al, 1942), while fat mass has a density of 0.900 g/cm^3 (Keys and Brozek, 1953).

The density of the body equals its mass per unit volume, therefore determination of B.D. requires the measurement of body volume and body mass which is easier to measure. Body volume is equal to the difference between body mass in air (BMa) and when totally submerged in water (BMw) (the body submerged in water is pushed up by a force equal to the volume of the water it displaces), corrected for the density of the water at its temperature (Dw). However, before the density of the body tissue can be calculated, the residual volume (Res. vol.) which refers to the volume of air in the lungs and gastrointestinal tract must be deducted from the whole body volume (B. vol). Then, the proper equation would be:

Body fat content can then be estimated according to Siri's equation (1956):

$$B.D. = \frac{B.M_a.}{B.vol. - Res.vol.}$$

where

$$B.vol. = \frac{B.M_a. - B.M_w.}{Dw}$$

Body fat content can then be estimated according to Siri's equation (1956):

$$Fat[\%] = \left(\frac{4.95}{B.D.} - 4.50\right) \times 100$$

The volume of air in the lungs is either predicted from height and age or vital capacity, or simultaneously measured, by re-breathing of marker gas until a steady state is achieved. The air in the gastrointestinal tract ($\simeq$ 100 ml) is not necessarily measured, because it is considered to be so small that its variations are well within the basic error of the method (Durnin & Satwanti, 1982).

The reproducibility of this technique has been tested by Durnin and Taylor, 1960 who found that the standard error of a single observation was 0.0023 units of density so that in 90% of the cases the error is likely to be $\pm$ 0.0046 g/cm^3.

observation was 0.0023 units of density so that in 90% of the cases the error is likely to be $\pm$ 0.0046 g/cm^3.

5.1. Biological variation in the density of the fat free mass and lean body mass.

It was already seen in figures 3.1 and 3.2 that the components of the FFM and of the lean body mass (LBM) may vary.

Bone mineral content (BMC). One of the main causes for a shift in the density of the FFM can be attributed to the amount of mineral in the skeleton, because of the high density value of mineral. It has been found that males have more mineral than females and that young subjects more than the elderly; racial differences have also been found.

Some studies have shown that the BMC of the radius, the third metacarpal, humerus, femur and vertebra, measured by photon absorptiometry techniques, increases from early childhood to reach a peak at about 20-25 years in males and 30 years in females; it remains constant up to the age of about 50-60 years in males and 40-50 years in females, and then falls gradually more for females, and it stabilizes again from age 65-70 up to about 80 years. The main difference between sexes is that women lose BMC, to a larger extent and earlier than men, process which accelerates with menopause (Sorenson et al, 1968 and Smith et al, 1969).

Skeleton cadaver analyses have shown differences in the density of various segments of bones (Trotter et al, 1959 and Baker & Angel, 1965) in the same dissected cadavers of individuals over 45 years. Negro male cadavers were found to have higher bone density values than White male cadavers; however, no such racial difference was found in females by Baker & Angel, 1965. BMC was lower for females and so the density of almost all bone segments was higher in the males; a decrease of density with age was also found.

Durnin and Womersley, 1974 have stated that the decrease of the estimates of the BMC with age cannot be given for true on these basis, as the rates of demineralization of bone at certain selected sites may not reflect the rate of the skeleton as a whole. However, they have calculated that a fall in the mineral of the body of the order of 8 to 15 % (the estimated decrease in men between the ages 45 to 75 years) up to 30 % (the maximum decrease reported for women) is equivalent to a fall in density of the FFM of about 0.003 to 0.012 g/cm^3.

Being the mineral density as high as it is, 3.1 g/cm^3, any change in the amount of what it is assumed (about 7 % of the FFM), produces an important change in the overall density of the FFM.

In his review on B.C. Lohman, 1981 reported the following standard deviations of fat% associated with biological variation in the density of the FFM. Variation in water makes the largest contribution to estimates of variability in density and fat content. Variations were calculated assuming the values estimated by Siri, 1956; i.e., for water a value of 2%; for protein/mineral ratio the lower range value of 10%.

Source of variation	Fat [%]	Density [g/cm³]
Water content	2.7	0.0057
Protein/mineral ratio	2.1	0.0046
Mean fat content of obesity tissue	1.8	0.0039
Obesity tissue density	0.5	0.0011
Mean fat content of reference man	0.5	0.0010
Total	3.8	0.0084

Figure 3.3. Variability of fat% associated with biological variation in the density of the FFM.

Lohman has also reported on the biological variation in the density of lean body mass (LBM) that was estimated by Bakker and Struikenkamp, 1977 to be of about 0.01 g/cm³. The first source of variability was found to be water with a standard deviation (S.D.) of 8%; second the skeleton with two sources of variability which are: estimates of variation in the skeletal fraction of LBM in the order of 17 to 23%, corresponding to a change in density of 0.12 g/cm³; and estimates of variation in the density of skeleton from 1.22 to 1.30g/cm³ corresponding also to 0.12 g/cm³; the S.D.s were 3% for both sources. The third source of variation is the fat-free adipose tissue content included in LBM; for very obese subjects, the calculation using a S.D. of 2%, is a reduction in the density of the LBM up to 0.006 to 0.007 g/cm³ . The last source of variability is the lipid content of the LBM estimated to range from 2 to 5%, with a S.D. of 15% which change the density by 0.006 g/cm³. All together give a total variability of 0.0094 g/cm³.

This biological variation in the LBM density corresponds to a S.D. of 3.4 % in the estimated fat content and is similar to that found by Siri, 1961 associated with the uncertainty in the density and chemical composition using reference man and fat free body.

Adipose tissue, not fat alone, have been reported to increase with age (Durnin and Womersley, 1974). Adipose tissue comprises about 64% fat, 22% cell residue and 14% extracellular water (Brozek et al, 1963). If the cell residue, in turn, is essentially the same as lean tissue and consists of 70% water, the composition of obesity tissue is then 62% fat, 31% water and 7% protein (Siri, 1956). An increase in adipose tissue, will tend to diminish the density of the FFM because the extra cell residue and water of the adipose tissue have together a density of about 1.047 g/cm^3 that will become part of the FFM. Durnin & Womersley, 1974 calculated for their subjects that the observed mean changes in adiposity with increasing age would bring about a mean reduction in the density of the FFM of about 0.004 g/cm^3 in women and 0.003 g/cm^3 in men.

5.2. Residual Volume.

Measurement of lung residual volume (Res. vol.) is what makes the underwater weighing (UWW) densitometry method cumbersome and it is the biggest source of technical error for the determination of body density (B.D.).

Full expiration is desirable in order to have as little air in the lungs and respiratory passages as possible, because it makes the possibility of error to be smaller; however, on this condition subjects are able to be under water only for a few seconds and the time to register the weight is often insufficient. However, it is important to measure this residual air in each UWW measurement because the volume of the full expiration is not always the same and so the weight underwater varies according to this volume.

Rahn et al, 1949 found that at the end of the third expiration, taking three seconds to complete each cycle of inspiration-expiration, the gases in the respiratory passages plus in the lungs and in the anaesthetic bag, were in almost complete equilibrium. These authors found that the nitrogen (N) in the alveoli is normally close to 80.0 % of the dry gas whereas that of the anaesthetic bag containing oxygen has been found by Durnin and Womersley, 1974 to be about 0.8%. The total volume of the system will be equal to the Res. vol. in the lungs (R) plus the volume in the anaesthetic bag (V); the volume of the bag before re-breathing and the N content of the bag before and after re-breathing are known. R can be calculated since it is a closed system and the total N at the beginning and at the end will be the same:

N content of the lungs and bag before re-breathing = N content of whole system after re-breathing.

$$\frac{80}{100}\times(R+25)+\frac{n}{100}\times(V)=\frac{N}{100}\times(R+V+25)$$

where:
80 is the N content in the alveoli
25 is the volume of the two-way tap
n is the volume of nitrogen accompanying the oxygen in the anaesthetic bag.

The equation condenses to:

$$R=F\times\frac{N(V+25)-2000-Vn}{80-N}$$

where
F = correction factor to account or atmospheric pressure, body and spirometer temperature, the saturated vapour pressure of water in the lungs and in the spirometer.
Vn = Vol. of N in the anaesthetic bag
V = Vol. in the anaesthetic bag

A biological source of error that has been considered is the amount of gas present in the gastrointestinal tract. This gas content may be in the range of 50-300 ml (Bedell et al, 1956). However, Durnin & Satwanti, 1982 measured body fat from body density on 15 adults after food and carbonated drink consumption finding that the estimate of fatness changed at the most 1.5% and concluded that the variations observed are well within the basic errors of the method.

6. TOTAL BODY WATER (TBW).

The assumptions for the determination of FFM and the relative proportion of fat from the measurement of TBW are that FFM has a fairly constant water content of about 72-73% and that chemical fat is anhydrous.

6.1. Measurement of TBW.

This compartment is usually measured by the isotope-dilution method which consists in administrating a tracer dose of isotopical labeled water, which will evenly mix throughout the total water pool reaching equilibrium with body water within 2 to 8 hours. The principle of these methods is that when a known amount of a tracer substance is

injected into an unknown volume of a substance with which it mixes uniformly and completely, the final concentration of tracer provides a measure of the unknown volume.

The tracer of choice should be non-toxic, achieve a rapid and even distribution throughout all body water compartments, not be metabolized or excreted and be easily and accurately quantitated (Halliday, 1985).

The more widely used agents for measuring body water are antipyrene and water labelled with either deuterium (D_2 or $^2H_2$), tritium (^3H) or the heavy stable isotope of oxygen (^{18}O). Tritium has the advantage of being easily measured by scintillation counting; however, it is radioactive and has a long half life of about 12 years, whereas D_2 and ^{18}O are naturally occurring, stable and non-radioactive isotopes and are therefore preferable for use in man. For antipyrene, the fact that has to be given intravenously makes it inconvenient for the subjects, whereas labelled water can also be given orally and assayed in any sample of body water such as urine, saliva or plasma.

The analytical techniques for the estimation of the isotope content of aqueous media are various. i.e., scintillation counting for tritium. The falling drop method, freezing point elevation, infrared spectroscopy, gas chromatography and mass spectrometry for deuterium. Infrared absorption and mass spectrometry for ^{18}O (Halliday, 1977). The method and tracer to use depends mainly on equipment availability.

The possible body fluids to measure TBW are saliva, urine, plasma, tears, etc.. The most convenient body fluid of choice has been found to be saliva; it is preferently chosen as its collection is the least inconvenient, traumatic and/or stressful for the subject. The time required for deuterium to achieve equilibrium in the salivary glands is about three hours after isotope administration; when urine is used this period is about 5 hours. Then, the use of saliva minimizes the time the volunteers require to wait to have their samples taken. However, since naturally, there exist certain concentration of isotopes in the body it is essential to compare the deuterium of the saliva before and after administration of the label if accurate estimates of TBW are to be obtained (Halliday, 1985).

The concentration of the tracer in a sample of body water either saliva, urine or plasma, once equilibrium has been reached (or the increase of concentration if the tracer were already present in the body) provides an estimate of TBW; the calculation of its volume is based upon the simplified relationship:

$$C_1 V_1 = C_2 V_2,$$

where:

C_1 is the concentration of tracer given;
V_1 is the volume of tracer given;
C_2 is the final concentration of tracer and
V_2 is volume of TBW.

and

$$V_2 = \frac{(C_1 V_1)}{C_2}$$

On the assumption that the FFM is 73% water, body fat content can be calculated from TBW, as follows:

$$Fat = Body\ mass - \frac{V_2}{0.73}$$

$$Fat\% = 100 - \frac{(V_2)}{0.0073 \times Body\ mass}$$

In practice body water is continually changing as water is lost in the urine and by evaporation from the lungs and skin or gained from food and drink. Therefore, some degree of standardization is necessary to avoid extremes of either overhydration or dehydration, and measurements are usually carried on in the morning in the fasting state, with the subject avoiding eating and drinking during the equilibration period (Lawrence, 1990).

A related problem concerns the continuous loss of water and therefore tracer from the body during the period of equilibration, and how this should be taken into account. The following equation allows calculation of the errors likely to be introduced into the measurement of TBW by loss of water and tracer during the equilibration period.

$$\text{calculated TBW} = W_r + R * W_l$$

where:

W_r = weight of water remaining in the body at equilibrium,
W_l = weight of water lost from the body during equilibration,
R = ratio of the concentration of tracer in W_r divided by the concentration of tracer in W_l.

If R were equal to 1, calculated TBW would equal $W_r + W_l$, i.e., TBW at the time of dosing. If, on the other hand, R were 0 (water lost during equilibration was unlabelled), calculated TBW would equal W_r, i.e., TBW at equilibration. From a physiological point of view, the difference between these two estimates of TBW is irrelevant.

In practice, R will lie somewhere between 0 and 1 (probably closer to 1), because the concentration of tracer in water lost during the early phase of equilibration will be lower than that at equilibrium, and calculated TBW will therefore be slightly lower than $W_r + W_l$.

Assuming the following: a) an evaporative water loss of 200 g during the 3 hours of equilibration, b) a basal urine production of 100 g / 3 hours and c) that R = 0.8 then, calculated TBW would, in a typical young women, equal 99.8% at dosing. Under reasonably standardized conditions, therefore, losses of water and tracer during equilibration are likely to be of little practical significance and can be ignored (Lawrence, 1990).

The dose must be large enough to produce a readily measurable increase in the isotope concentration in the body, i.e., C must be large in relation to the precision with which it can be measured. Precision can be assessed by analysing samples in duplicate. Lawrence, 1990 found that the mean difference between the first and second duplicates in one set of analyses was, for 33 samples, 0.06 $\pm$ 1.8 ppm (1 S.D.). In this case, the difference between samples can be measured with a precision of $\pm$ 1.8 ppm. For the analytical error to be < $\pm$ 1% it is therefore desirable that C should be at least 100 * 1.8 = 180 ppm, at least when pre- and post- dose samples are being measured once.

The total analytical error of the mass spectrometer procedure to measure deuterium is estimated to be $\pm$ 0.5 %, while the overall error in the TBW determination, not counting a systematic error because of hydrogen exchange, is stated as $\pm$1%. Measurement of TBW is considerable more reliable than is that of the extracellular fluid space because tracers do not all mix freely within the same fluid volume (Siri, 1956).

Labeled water has been found to overestimate TBW by about 0.5-2%, because of hydrogen exchange; the precise value depends upon the relative amount of lean tissue, thus the error relative to body mass would be smaller in obese and edematous persons than for lean

subjects (Siri, 1956). The overestimation of TBW should then be taken into account in the calculation to avoid underestimation of fat%.

6.2. Other methods to estimate TBW.

Combined Methods: If water and density are both measured, a 3 compartment system may be established; the fat free residue consisting of protein and mineral, may be termed non-fat solids. Siri, 1961 realized that the basic problems with B.C. measurements are more a consequence of incompletely defined models rather than the techniques for measurement, he suggested that there would be a considerable profit in combining independent measurements of density and TBW because the magnitude of the error could be lower than by estimates from density or water separately. It also gives a more confident measure of the absolute proportion of fat since the combined methods leave less chances for unaccounted variations in the body's components. Another important consideration is that this method is independent of the state of hydration, then it is equally valid for normal subjects and for patients with changes in TBW.

Newer methods: More recent developments (late 1960's) to measure TBW include: total body electrical conductivity (TOBEC) and impedance. These variables are primarily related to the body's water or electrolyte content. As methods for predicting TBW they relay on the development of appropriate regression equations, the impedance method offering the advantage of portability and much lower cost.

Technical imprecision can be more easily measured than the errors that can be achieved by using the assumption of a constant relationship between the components of the FFM.

6.3. Biological variation in the amount of water of the FFM.

Water is usually the largest component of B.M.; typically, a man weighing 70 kg will contain about 42 kg of water, or 60% of B.M. (Garrow, 1982). It constitutes the largest fraction of basic material of cells and the external environmental medium for the cell that form an integral part of the animal. The function and distribution of water define the two major fluid compartments which together constitute the TBW: the intracellular and the extracellular water.

The amount of water relative to B.M. in the normally hydrated body is dependent primarily upon the quantity of depot fat and diminishes with increasing obesity. In the leanest possible body, without storage fat, TBW constitutes about 72% of B.M. and for the most obese body, water can constitute 38% of B.M..

The dependence of water on fat is poorly understood. Adipose tissue is known to contain 10-30% water, but the exact proportion is not known, nor is it certain that the proportion is constant for different degrees of obesity. Based on the 2 compartment system adipose tissue:lean body mass Siri, 1956 estimated that the value for the range of variation of the water of the LBM is ± 0.03 and that for the water of the adipose tissue could be about ± 0.05. He points out that these estimates are not the limits of normal hydration because the quantities that enter into the calculations are mere approximations; but it does, however, suggest the order of magnitude of variation in TBW that cannot be attributed to differences in fat.

Pierson et al, 1982 concluded from their own measurements of TBW in subjects in the age range from 20-80 years and from other studies, that the decline of TBW with age is consistent in both sexes and that the actual steepness of the slope varies directly according to the relative fatness of the population studied: there is an inverse relationship of fatness to TBW. However, the decline of TBW with age is apparent in both sexes even when the effect of increasing fatness is taken into account indicating an actual decrease in FFM.

Age and **sex**, have also an effect on TBW. Between birth and the first year of age, TBW decreases from 80% to about 60%. In men, there is a slight gain until the early twenties, when TBW begins to decrease slowly with age, surely due to the accumulation of storage fat during adult life. Women do not seem to gain water relative to body mass in youth but show a steady decrease during the life span; they also present lower TBW than men. Both these factors are attributable to differences in storage fat with age and sex (Siri, 1956). Pierson et al, 1982 found in a study on 58 subjects aged 19-80, that the amount of water of the B.M. is higher in males than females at all ages, related primarily to larger relative muscle mass, and lower fat content and that the rate of decrease with age in females is 0.36% per year and in males is 0.26 % per year.

7. TOTAL BODY POTASSIUM (TBK).

"On the assumption that the potassium content of the lean body mass is constant, it should be possible to estimate fat content in living man from a measurement of potassium-40 activity in the whole-body scintillation counter" (Forbes et al, 1961).

The principle underlying this method is the same as that of the densitometric (D) and the TBW methods of measuring body fat, which divides the body in two compartments fat and FFM. The amount of potassium of the FFM (K/FFM) is assumed to be about 60 mmol/kg for females (Womersley et al, 1972) and about 68 mmol/kg for males (Forbes et al, 1961) and the amount of K of the chemical fat is zero.

Then, if the amount of K of the .body is known, its fat and fat free (FF) masses can be derived.

7.1. Measurement of TBK. Instrument, Procedure and Calibration.
The naturally occurring radioisotope ^{40}K has a half life of 1.25×10^9 yrs. and is assumed, by virtue of its natural occurrence and long half life, to be equally distributed in all living biological material. It occurs in TBK to the extent of about 0.0118 % which emits γ-rays of energy 1.46 MeV which may be detected using sensitive low-background whole-body radioactive counters (Boddy et al, 1971). Since the ^{40}K isotope represents a constant proportion of TBK, it acts as a naturally occurring tracer and TBK may be determined comparing the count rate against that of calibration standards.

Quantitation of TBK requires specially constructed counters that consist of a large shielded room, to reduce background radiation from cosmic and terrestrial sources, containing a γ-ray detection systems connected to a suitable recording device. The detectors are of two types: large thallium-activated sodium iodide crystals, one or more of which are positioned near the subject, and large, hollow cylinders or half cylinders, the wall of which contain liquid or plastic scintillation material and into which the subject is placed so as to be completely or partially surrounded by the detector. The advantage of the crystal system include very good energy resolution and a low background rate.

Calibration of the instruments to measure potassium for use with humans must be performed. The administration of ^{42}K has been done to correct the main factor of uncertainty in these measurements which is the counting rate per gramme of potassium with body build. γ-rays produced by disintegration of ^{40}K are detected by scintillation detectors and these instruments must be calibrated individually. By adding a known amount of the radioactive isotope ^{42}K to the body on the assumption that it mixes uniformly with TBK, the calibration can be performed. This isotope is used because the body will not take up excess potassium and it emits γ-rays of similar energy as does ^{40}K.

Briefly, the calibration procedure consists in:
a) giving a known amount of the radioactive isotope to a subject (about 5μCi),
b) constructing phantoms (p) (containers with known amounts of ^{40}K (**^{40}K p [g]**) and ^{42}K (**^{42}K p [g]**) with same volumes of distilled water) and
c) measuring these isotopes in the subject (s) to know their γ-ray spectrum in counts per minute (cpm) before (**^{40}K s [cpm]**) and 24-48

hours after administration of ^{42}K (^{42}K s [cpm]) (when equilibrium is achieved).

d) The phantoms containing ^{40}K (^{40}K p [cpm]) and ^{42}K (^{42}K p [cpm]), are also counted to be compared.

After correcting for the amount of the ^{42}K excreted in urine and for the radioactive decay up to the time the subject is finally measured, the potassium content of the subject is calculated with the equation:

$$K_{[grams]} = {}^{40}Ks[cpm] \times \frac{K^{40}p[g]}{K^{40}p[cpm]} \times \frac{K^{42}p[cpm]}{K^{42}s[cpm]}$$

The second factor in the above equation is a constant. The third is called the **"g' factor"** and is a geometrical correction for the fact the γ-rays originating from the phantom are not counted with the same efficiency as those from the human body.

Boddy et al, 1971 carried out a calibration study, using the radio-isotope and the above mentioned procedure, in 69 healthy subjects of both sexes (30 females, 39 males) and found that the "g' factor" could be predicted from the following regression equation:

$$g'factor = (91.4 + 3.32W + 4.78H) \times 10^{-3}$$

where:

W = body mass, in kg;

H = height, in cm

Use of this equation makes it unnecessary to carry out the calibration procedure on each individual subject. Calibration of the instrument itself, using a phantom of known K content must be carried on frequently.

A common index of the sensitivity of whole body counters is the performance in measuring TBK. For a human subject containing 3600 mmol (140 g) TBK and a counting time of about one hour, the statistical C.V. is 2.1%, when extrapolated from a measurement made with a phantom. This sensitivity compares favourably with that of many shielded-room counters also using sodium iodide detectors (NaI) (Boddy et al, 1975). When the calibration factor is used, as in the present study, the standard error has been calculated to be about 3.9%; a change >4.6% in the measured TBK in the same subject, would be significant (Boddy et al, 1971).

7.2. Biological Variations in the amount of potassium of the FFM (K/FFM).

Potassium (K) appears in the body as an intracellular cation localized primarily in muscle tissue. The relation of K to FFM has been used as a valuable index, because of their close association.

Information on the K/FFM can be obtained from cadaver information and by the measurement in vivo of K and the FFM. Direct chemical determinations have been applied to only small numbers of cadavers; the results obtained are difficult to generalize and must be taken with caution. More information is available on the indirect determination of the ratio of K to FFM both measured by independent methods.

Variations in the ratio K/FFM are to be expected since both, K and FFM are prone to biological and technical errors.

Biological variations include among the main variables: age, sex, muscle, adipose tissue and bone contents. The composition of the FFM presents variations in the proportions of its anatomical and chemical constituents and it can be indirectly predicted from various independent methods with their own assumptions and technical errors. Variations in skeletal muscle alone, the richest tissue in K (mean range 92-102 mmol/kg), might be the main cause of variation of K/FFM.

Data on cadavers. Forbes et al, 1961 proposed the use of TBK content as an index of FFM based on results of chemical analysis of 4 human adult cadavers which revealed values of 66.5, 66.6, 72.8 and 66.8 K/FFM [mmol/kg]. The two first values are of two male cadavers analysed by Forbes & Lewis, 1956 aged 46 and 60 years, respectively. The third value is the value of a female cadaver aged 42 years analysed by Widdowson et al, 1951; it is interesting to note that this last value is the highest of all, this subject was described as being of a thin masculine-type, and was of lower body mass and taller than the average. The mean value of the four cadavers, 68.1 mmol/kg is the value suggested as being constantly related to FFM for both sexes and proposed to be used for calculations of body composition (B.C.).

There exist the value of another, fairly normal, male cadaver, aged 25 years, reported by Widdowson, 1951, with a ratio K/FFM of 71.2 mmol/kg.

These are the only analyses which have been carried out on individuals who were not suffering from diseases known to profoundly affect K metabolism.

Other workers have emphasized the relative constancy of TBK content in animal species when values are expressed on a fat free basis, though the absolute values are in some instances higher than

those for man. Forbes & Hursh, 1963 have compiled data on this matter; studies on rats has yielded mean values of 81 ± 4 and 73 ± 3 mmol/kg; for rabbits a range of values from 81-86 mmol/kg; for pigs a range from 71-76 mmol/kg, and a mean value of 69 ± 4 mmol/kg. Womersley, 1974 has completed these data with a study in pigs with a range of values from 63-77 mmol/kg and in dogs a range from 50-70mmol/kg.

In vivo Measurements. The amount of TBK can be determined by measuring ^{40}K by whole body external counting, the most used and common technique, but also exchangeable potassium (K_e) may be measured.

FFM may be derived by any of the available indirect methods; the most common being: densitometry (D), measurement of TBW, anthropometry, etc.

Following some studies concerned with physiological variations of TBK in man are presented. It shall be seen that age and sex are the main reported factors which determine the amount of K of the FFM; however, its variation depends upon the amount of muscle mass, fatness and other genetic factors such as height and the size of organs, mainly those rich in K.

a) Age and sex effect on K/FFM. Aging has been demonstrated to be related with a decrease in the total amount of K; the reported age for the start of K decrease and its rate varies among sexes and investigators.

Myhre & Kessler, 1966 measured in 100 males from 15 to 87 years, their body density by UWW and residual volume and ^{40}K by whole body 4π liquid scintillation counting. They found an increase in the difference between D and K methods in their estimates of body fat as proportion of B.M. with advancing age. They deduced that age is not a factor contributing to the difference between the estimates of fat obtained from these 2 methods, from 15 to 58 years but from 60 years the differences are greatly affected by age. In their discussion the authors say that the discrepancy between methods is probably due to the influence of an increase in the ratio of connective tissue to muscle with aging.

Shukla et al, 1973 measured TBK by whole body counting on 915 (56 females, 859 males) healthy adult (20-69 years) subjects and analysed the data with age and sex as variables. They also measured 10 subjects (age range at the start of the study: 25-40 years) at monthly intervals over a 12 year period. They found that the rate of

decrease in females was of about 6.6 mmol/yr (0.26 g/yr) and for males 14.5 mmol/yr (0.6 g/yr). The rate of decrease of K expressed per kg of B.M. was 0.13 mmol/yr/kg (0.005 g/yr) in females and 0.21 mmol/yr/kg (0.0083 g/yr) in males. A close agreement was found by these authors in the comparison of their results with the values obtained by other investigators, which are on the range of 0.14 to 0.24 mmol/kg (0.0055 to 0.0095 g/kg) in the age range 20-80 years. The 10 subjects measured over 12 years did not change B.M. over this period and presented a mean drop in TBK of about 102 mmol (4 g) for the 2 females (8.5 mmol/yr) and of 153 mmol (6 g) for the 8 males (12.7 mmol/yr); this decrease, the authors say, was within the statistical limit of counting error (2.5%) of the technique employed. The mean coefficient of variation (CV) for each individual over a one year period (12 measurements) was $\pm$ 5.6% for the females and $\pm$ 5.2% for the males. These results showed the same pattern as that of the first part of the present study.

Diurnal variation was also studied by Shukla et al, 1973 in 6 subjects that were on a fixed K diet (3.5 g/day), over a period of 10-15 days. The K content varied from SE: 16.1 to 26.3 mmol (0.63 to 1.03 g); this variability was also included in the counting error of the technique.

Novak, 1972 studied 305 women and 215 men, healthy, whose ages ranged from 18 to 85 years. He measured TBK by whole body counting of ^{40}K and from this measurement he derived FFM and fat% using the constant value of 68.1 mmol/kg of FFM and cell mass under the assumption that cells contain 3 mmol of K per gramme of nitrogen (N_2) and that the wet weight of cells equals their N_2 content multiplied by the coefficient 25. He found age to have a significant effect in the amount of K. Women were found to have the same amount of K up to the age of 55 years (about 2550 to 2650 mmol or 100-103 g), then a decrease for the next 2 decades of about 170 and 50 mmol, respectively, were noted. Men had a stable amount of K ($\approx$ 4100 mmol or 160 g) up to the age of 45 years, from there a decrease in the following three decades was seen: about 335, 170 and 440 mmol, respectively. Sex also had a significant effect on the absolute amounts of K and relative to B.M. at all ages. Males had higher values than females. Further calculations of percentage changes in relative amounts of TBK per kg of B.M. correspond in females to decreases of 5.4% between the ages 30-40 years, of 10.5% between the ages 40-50 years, and of 2.3% and 1.6% in the two following decades. For males, the decreases were of 4.6% between the ages 20-30 years; then for each decade, respectively: 1.5, 6.1, 3.4 and 9.2%. The magnitude of sex differences in relative values of TBK revealed that

females had on average about 83 ± 3.2% of males' average; differences were independent of aging. Fat, FFM and cell mass were also affected by age on both sexes. Fat increased with age and FFM & cell mass decreased concomitantly. The trends were similar for both sexes but the magnitudes were different. These changes were in fairly agreement with the decrease in K at the same age groups.

Physiological variations of TBK with sex, age, height and fatness have been described by Pierson et al, 1974. These authors measured TBK in 3,083 healthy subjects from ^{40}K by 4π whole body scintillation counting and estimated body fat and FFM by anthropometric measurements using the method proposed by Steinkamp et al; age ranged from 4-90 years. The sum of biological and instrument variances measured beforehand in 13 volunteers at weekly intervals (body mass 44-87 kg; height 140-189 cm; TBK 1820-4850 mmol) ranged from 1.2 to 4.8% (S.D.) about the mean, this deviation applies to 36-170 measurements. The authors expressed their results of K using as normalizing variables (denominators): height (ht), body mass (B.M.), body surface area (BSA) and FFM, as the mean standard error about the mean for age groups. Their results showed a sex difference in the amount of K related to ht, B.M. and BSA from about 12-14 years of age; TBK was found to be constant from age 20-45 years for females, then declined 0.23 mmol/kg of B.M. per year (0.009 g/kg), averaging 0.7% per year. For males, an increase of K to a peak of 53.8 mmol/kg (2.1 g/kg) at age 20 and a decrease thereafter at the average rate of 0.26 mmol/kg per year (0.01 g/kg) was seen. The K/B.M. ratio showed variations of 2.3% for females and 1.4% for males. A substantial decrease in K was found to occur over the 20-80 age range (39% for females and 33% for males), suggesting that two processes, dilution of K concentration by added fat and reduction in muscle mass, are both occurring. The K/ht ratio showed a mean variation of 2.5% for women and 1.8% for men; the secular changes in K affects males more than females. The ratio K/FFM showed variations that ranged from 1.0 to 2.8% in females and from 0.9 to 2.2% in males. For 308 females figures of 57.8 mmol/kg (2.3 g/kg) and for 182 men from 21-40 years of 67.8 mmol/kg (2.6 g/kg) were measured. An effect of fatness on TBK was found: male subjects with less than 25% fat had higher K/FFM ratios than those with higher values of fat proportion. In their discussion the authors make note that a variance of ± 10-15% in TBK within each sex and age groups is quite similar in various reported studies and that fatness is the main fact determining this variance. Referring to the fact that FFM gave the lowest variance (10%) among the denominators examined the authors say that it was not possible to reduce it further and give two apparent

explanations: the fact that the method for measuring fat may include an error of 10% in an individual subject and that the activity level of 'normal' subjects varies widely.

Years later, Pierson et al, 1982 in a study of 58 adults (28 females 30 males), age range 20-80 years, showed significant age regressions in both sexes for TBW and intracellular water (ICW), and for sodium and K when referenced to TBW. The rate of decrease of TBW expressed as a proportion of body mass was, for females 0.36 and for males 0.26% per year; ICW is the compartment that primarily changes: it decreases steeply with age, the decline being 50% steeper in women (0.46% per year) than in men (0.30% per year). The ECW/ICW may increase with age, depending on fatness. The highly significant decrease of TBK with age in both sexes parallels the decrease in ICW. The slope as % per year of TBK per kg of body mass found was, for females -0.55 and for males -0.43%. The rate of decline expressed per kg of TBW was for females -0.29 and for males -0.22%. Combination of these findings confirms previous studies that body cell mass decreases with age. The concentration of intracellular potassium (K_i), calculated from the measurements of TBK and ECW, was also found to decline with age, suggesting that the shrinking lean body is also losing K_i, but this finding was not well established. This decrease with age did not occur in one constantly exercising man, so long as the exercise level was maintained, but it has been consistently shown to apply to larger normal human populations. This decrease because of a relatively greater loss of skeletal muscle, higher in K_i. Based on the results in other studies in which rats did not lose K, lean body, or skeletal muscle and did not gain fat, over a full life span, the authors say that this trend is not a programmed and inevitable mammalian sequence. However, all studies in man confirm declining K^+ with age regardless of the normalizing denominator used. These authors concluded that the usual wide ranges of the values for normal body composition which derive from biological variation (SD $\pm$ 7% of mean) may become narrower if body water rather than body mass is used as the normalizing variable.

The reasons given by all these authors for the trend of TBK to decrease with aging in healthy men and women are: by atrophy of cellular mass either by disuse or as a consequence of the physiological aging process. Because muscles contain approximately 70 % of the TBK, a decrease in the relative amounts of K suggests less muscle mass and a replacement of tissue high in K by tissues with very low K content; such tissues could be connective tissues and fatty tissues, both of which are essentially free of K (Novak, 1972).

To give a quantitative idea, Myhre & Kessler, 1966 reported values of the K content of 2 forms of connective tissue: cartilage and tendon, of 178 and 120 mmol K /kg dry weight, respectively and of lean muscle of 487 mmol K/kg dry weight. The decrease of K concentration with age, may also result from a decrease in K concentration in muscle, a decrease in the non-muscle K compartment, or from a combination of all these effects (Pierson et al, 1974). It is a common feature the trend found in the increase in body fat and/or decrease of FFM or muscle mass with aging (Novak, 1972).

The different rates of decrease between sexes, are attributed to different physiological events: in females to an increase in body fat in excess of muscle, continuously during the growth periods as well as during aging. In males, muscle is added in excess of fat up to 18 years, after this age, fat increases and lean body mass, i.e., K decreases (Shukla et al, 1973 and Pierson et al, 1974).

Loss of skeletal muscle (which is approximately 80% the ICW by weight), and its replacement by adipose tissue (about 3% of which is ICW), is a potent explanation of the decline in ICW in both sexes (Pierson, 1982).

The sex difference has suggested to different investigators that even after body fat is subtracted, a higher K concentration in the FFM is seen in men.

Delaware and Crenier, 1973 measured, on 296 young (mean age 20 years) subjects (161 females and 135 males) in good health, from the same ethnic origin, all engaged in some physical activity although not competitive, TBK by a whole body counter and determined FFM by TBW (tritiated water dilution) and anthropometry. They found the mean ratio K/FFM [mmol/kg] to be 56.8 for females and 62.7 for males; the margin of variation around the mean values was wide, i.e., for females from 49-78 and for males from 40-80 mmol/kg. These authors calculated the K/FFM [mmol/kg] ratio from publications concerning the ± 20 year old group, which are: Meneely et al, 1962: women 44.5 ± 3.2, men 57 ± 5.8; Anderson & Langham, 1965: women 44.4, men 54.4; Oberhausen and Onstead, 1965: women 42.3, men 55.1; Krzywicki et al, 1968: men 55.6 ± 6.2; Novak, 1970: women 44.1 ± 5.2; Cohn & Drombrowski: men 49.5 ± 2.7; Boddy et al, 1972 women 44.5, men 56.

In a study on 10 females and 10 males in which TBK was measured by whole body counting and FFM derived from 4 different techniques: height and B.M., skinfolds (SkF), density (D) and TBK using the equation of Boddy et al, 1971, Womersley et al, 1972 found, significant differences between sexes; for females a mean value

estimated by the four methods [mmol/kg] of 59.7 (range 57.8-62.8) and for males 66.4 (range 65.6-67.3).

Womersley et al, 1973 have compiled the ratio reported by several workers or derived it themselves. The values of 12 studies for females range from 48-63 and for males from 56-70 mmol/kg. The values of K/FFM are consistently higher in males than in females and in those studies in which both males and females were included significant differences have been shown. Later studies show that the values are in the same ranges just mentioned. For instance, Sjöström et al, 1986 deduced a K/FFM of 62 mmol/kg from measurements of computed tomography and TBK in 12 healthy women.

Some controversy on this matter has been put forward by Burkinshaw & Cotes, 1973 who did not found such differences between sexes. They studied 36 females and 31 men and divided them to show that the main reason for the differences found by other authors is the physical activity that the subjects performed. Sedentary females and males did not have significant differences (females 58.3 $\pm$ 3.1 males 58.8 $\pm$ 3.3 mmol/kg; $p < 0.001$) and a difference was seen between the group of males that took regular exercise with all more sedentary groups (active men: 64.3 $\pm$ 3.7 mmol/kg).

b) Physical type effect, i.e., muscularity, on K/FFM.

Variations between subjects of different sex, age, physical activity and B.C. have been studied by Womersley et al, 1976 who did a study to assess the differences between groups of subjects in the mass and composition of the FFM and thereby to derive appropriate factors for estimating body fat and FFM. There were studied 43 women and 36 men, deliberately chosen to represent a variety of physical types; these were University technicians, students or staff forming the 'young sedentary' women and men groups; sports men engaged in competitive weight lifting, basket ball and putting the shot for the 'muscular men' group; sports women representing the University at hockey, squash or putting the shot for the 'muscular women' group; and normal obese subjects of various ages to form the groups: 'younger and older obese' and lastly, normal older individuals to form the 'older non-obese' groups. Measurements of body density (B.D.) and of TBK were performed.

They found greater mean values of FFM obtained by D and of the amount of TBK for the muscular compared to the sedentary groups; i.e., for females 50.3 vs 41.7 kg of FFM and 3290 vs 2500 Kmmol and, for males 68.7 vs 57.7 kg and 4760 vs 3790 mmol, respectively. Males were of similar height but muscular females were on average 9 cm taller than the sedentary group. Also found were considerable greater

values of FFM and K of younger obese women (51.0 kg and 3150 mmol) and men (73.1 kg and 4710 mmol).

The use of conventional values of the K/FFM, i.e. 60 and 68 mmol/kg for females and males, respectively, to predict fat and FFM, gave values that were significantly different between the D and the K methods for the groups of muscular and younger obese women and sedentary young men; the other groups presented non significant difference between techniques.

Based on the knowledge of the basic assumptions about the density and the normal amount of K content of the skeletal muscle as part of the FFM, the authors proposed the following figures for the density [g/cm^3] and the K [mmol] content of the FFM for women and men, respectively:

	Females		Males
- for young sedentary:	1.100 & 60	and	1.105 & 67.
- for young muscular:	1.090 & 63	and	1.095 & 69.
- for young obese:	1.093 & 60	and	1.100 & 64.
- for older non-obese:	1.090 & 58	and	no suggestion
- for older obese:	1.087 & 55	and	1.098 & 62.

The authors explain about these figures that they are not intended to remain as definitive but just as tentative until more subjects are investigated in each group.

Besides true population variations in the results of measured TBK, differences due to the use of different whole body counters and calibration procedures should be taken into account (Pierson et al, 1974; Shukla et al, 1973).

From these studies it can be summarized that TBK varies with age, a natural occurring process that causes changes in B.C. during the life span. Sex difference is usually explained because of a different amount of muscle, the richest tissue in potassium, but this effect is not fully understood. Health status and the level of physical activity may alter the normal trend and amount of potassium.

c) Amount of potassium of individual body tissues. Genetic variations.

Variations in K/FFM between or within subjects may be induced or naturally occurring (genetic). This can be better explained by the fact that the individual body tissues differ in K content. Forbes and Hursh, 1963 reported data on the K content of various tissues (in

animals and man) on a fat free basis; for muscle (rat) 99 mmol/kg; for liver (rat) 95 mmol/kg; for brain (rat) 101 mmol/kg; for adipose tissue 56 mmol/kg; for marrow-free bone (rat and man) about 20 mmol/kg. Size variations between individuals include bone and adipose tissue. Since these tissues have a low K content, subjects with relatively high proportions of bone or adipose tissue may have a lower K/FFM than a light-boned or a thin one. Of course, natural high amounts or induced shifts in muscle mass will be more important because of its high K content. Also, genetic variations in the size of any of the K rich tissues between individuals will bring about variations in the K/FFM.

d) Illustrated example of the effect of changes in body composition on TBK/FFM.

If theoretical calculations were to be made on variations in muscularity and adiposity, it shall be needed the K content of each tissue, the amount of tissues gained or lost and the amount of fat of the adipose tissue. Variations in body composition within a subject are difficult to calculate on theoretical grounds because a complete re-arrangement of the body components will take place; but let's just illustrate the variation in the K/FFM that would cause the gain of 5 kg of muscle mass and the lose of 5 kg of adipose tissue, as two separate events.

This example is illustrated with an hypothetical subject, a male weighing 70 kg, composed of 15% fat and 59.5 kg FFM. Because of the performance of physical exercise he has increased his muscle mass 5 kg. Under the assumption that TBK/FFM is 68 mmol/kg, his TBK is 4046 mmol; an increase of 5 kg of muscle containing 99 mmol/kg, yields an increase of 495 mmol. If his FFM increased from 59.5 to 64.5 kg now he has (4046 + 495 = 4541 / 64.5) 70.4 mmol/kg, then the K content of his FFM has increased by 2.4 mmol/kg.

If instead we assume that this subject has lost 5 kg of adipose tissue, assuming that adipose tissue contains 80% fat, he has lost 1 kg of fat free adipose tissue with a K content of 56 mmol/kg. This yields an increase in the K content of his FFM of only 0.2 mmol/kg.

As it can be observed the muscle mass produces a significant shift in the K/FFM, whereas adipose tissue changes this ratio insignificantly.

8. SKINFOLD THICKNESS.

The simplicity and accessibility of skinfolds (SkF) makes this method ideal for studies in which there are not laboratory facilities, but even when there are, it allows comparisons with other methods. It is cheap, easy and thus ideal for field and clinical studies.

The principle underlying this method is that the quantity of subcutaneous 'adipose tissue plus skin' at particular sites may be related to total body fatness.

The use of SkFs to objectively predict indirectly B.C. in terms of fat mass and FFM, is a great discovery (see Rahaman, 1966 and Womersley, 1974), since it is only necessary to measure a fold of skin plus its adjacent adipose tissue, with an easy to manipulate and economic instrument, to obtain the quantity of subcutaneous fat at particular sites.

Although the measurements need some skill to assure inclusion of all the adipose tissue excluding the muscle mass and attention should be paid to the specific description of the sites of measurement, once properly trained, the performance is quite simple.

8.1. Inter- and intra- examiner variability.

The inter-examiner error of this method has been studied by Womersley & Durnin, 1973. Three observers, with different degrees of experience, measured SkF thickness at 4 sites (biceps, triceps, subscapular and suprailiac), in 23 women and 27 men, young and non obese, on 5 occasions on average, over a period of one month. The mean S.D. for the sum of 4 SkF of repeated measurements calculated for individual subjects, i.e., reproducibility of the measurements, showed values for males, of 2.3 mm, by the most experienced observer, and 2.1 & 2.0 mm for the other 2 inexperienced, and for females 3.1, 3.7 and 3.6 mm, respectively. Thus, the measurements, made by the three observers were about equally reproducible. Using the results of the measurements on the right side of the body of this study, the C.V. were calculated to be, on average of the 3 observers, 7.5% for females and 6.7% for males and the mean proportion of fat would vary from 24 to 26% for females and from 13 to 14.5% for males. This means that the estimation of fat made by this 3 different observers would lead to a maximum difference of 2.0%. The authors found a significant difference of the mean values on both sides of the body between observers, but no constant trend was found in any observer. An even greater variation was found by these authors when, besides differences found by the 3 observers, those derived by the use of 3 different calipers were added up. The maximum difference they found between observers -that is the lowest reading by any observer and the highest reading by any observer- in the estimation of fat content was

of 5% of the body mass. In their discussion the authors express that usually the extreme range of fat content was much smaller than this but that even these maximum variations have minor consequences, at least in field work. Therefore, they conclude that variations in SkF thickness due to different observers, experienced and inexperienced, using the 3 different calipers, were not likely to influence critically the results obtained.

Intra-examiner error of SkF measurements was carried on by Lewis, 1990 at Durnin's laboratory. She measured the 4 SkF thicknesses in 13 young females and males, twice with a week difference; the mean fat% difference was 0.2 % (SD$\pm$1%).

With this information it can be said that, although there can be statistically significant differences in the total sum of SkF between observers, the amount of the difference may have only a slight influence on the prediction of total body fat content in the subject when measurements are carried on by different observers but practically no effect when measured by the same observer with the same caliper.

An important recommendation made by Womersley & Durnin, 1973 regarding the reproducibility of SkF measurements when different observers or calipers are used, is that it is preferable to measure all four SkF rather than single sites.

Also important in diminishing technical variations is to periodically calibrate the pressure exerted by the SkF calipers to a standard pressure and probably also to standardize the length of time for which the SkF is to be compressed, specially when not so experienced measurers perform the measurements.

8.2. Biological variations.

Variations due to biological sources also exist, most of which would be included within the assumptions of this method.

The SkF method relies on assumptions at various steps that range from the caliper reading to total body fat, and are the following:

- the proportion of subcutaneous to total body fat is relatively constant or at least predictable over all ranges of body mass;
- the sites selected for measurement represent the average thickness of total subcutaneous adipose tissue;
- there is a constant, or at least predictable, distribution of adipose tissue between subjects,
- the compressibility of the skinfolds is constant;
- the thickness of skin is negligible or at least constant among skinfolds and subjects, and

- there is a constant fat fraction in adipose tissue.
(Durnin & Womersley, 1974 and Clarys et al, 1987).

Compressibility of skinfolds. The thickness of a compressed double layer of skin plus subcutaneous adipose tissue should be representative of the uncompressed double layer of subcutaneous adipose tissue. Mean SkF compressibilities for different samples range from 16-51%. In the Brussels cadaver study (B.C.S.), the mean compressibility for 13 cadavers was about 53% (38-69%); differences were observed at different sites that are commonly selected for prediction of adipose tissue. Wide differences in SkF thickness among two cadavers with similar adiposity and different compressibility showed that the **largest error** in fatness estimation are compressibility variations (Clarys et al, 1987).

Durnin & Womersley, 1974 reported on various investigations using X-radiography as standards for comparison of the SkF against calipers exerting a pressure of 9.8 g/m^2, that have shown that SkF compressibility diminishes with age (Hammond, 1955; Garn, 1956; Garn & Gormon, 1956 and Brozek & Mori, 1958) independently of the thickness of the SkF. Also, it has been suggested that this decrease may be due to a decrease in the water content of the tissues present in the SkF (Brozek & Kinsey, 1960).

Skin thickness and distribution. The B.C.S. showed that skin thickness is different at various SkF sites measured and may have an important contribution in the assessment of adipose tissue (mean value 16.5 range 8-28%) and that males have a thicker skin than females; but, as it is generally of the order of a few millimeters, it would appear that the effect of skin would be most marked at those sites and in those subjects with little adipose tissue (Clarys et al, 1987).

Distribution of subcutaneous adipose tissue. The SkF method is a way to directly measure the adipose tissue situated subcutaneously. A large fraction of the store body fat in humans, about 50% or more, is situated in this location (Edwards, 1950); however, variable amounts have been reported between individuals.

The distribution of fat in the body is not equal in different zones, between individuals and within individuals at different ages; then, the sites chosen should prove to be representative of the subcutaneous adipose tissue of the whole body. Different authors utilize those sites which, based on their experience on the subjects they measure, are the most suitable depending on sex, age, physical characteristics, and ethnicity. Durnin & Rahaman, 1967 and Durnin &

Womersley, 1974 have chosen 4 sites: biceps, triceps, subscapular and suprailiac, which are the most convenient for the subject and the easiest to perform by the measurer. Clarys et al, 1987, from the results of the B.C.S., reported on the unexpected finding that the lower limb sites best predicted subcutaneous adiposity.

Amount of fat of the adipose tissue. The fat content of adipose tissue has been usually reported to range from 60-85% and the data on cadavers have confirmed the inverse relationship between water and fat with increasing adiposity and it has been estimated that the variation in the amount of water and fat% is of about 20%. Thus, two identical thickness of adipose tissue may contain significant different concentrations of fat (Clarys et al, 1978).

Ratio of subcutaneous (or external):intra-abdominal (or internal) adipose tissue. The reported external to internal (ext:int) fat ratio is variable and controversial (Womersley, 1974; Brown & Jones, 1977; Lohman, 1981). Age, sex, degree of fatness and measurement technique have been found to affect this relation.

Durnin & Womersley, 1974 found differences in the intercept of the regression lines relating SkF thickness and body density (B.D.), with aging and between sexes; the calculated decrease in the density of the FFM for the superadded adipose tissue and the decrease in mineral with aging could not explain on their own the above results; then, the difference was explained by a concomitant decrease in the proportion of fat situated subcutaneously with increasing age. For women, they concluded that there is more internal than subcutaneous fat (the ratio ext:int fat is lower), because a given SkF corresponded to a considerably lower B.D. than in men; the same was also concluded for older subjects; for females the value for B.D. which corresponded to a given Σ4SkF decreased by about 0.004×10^3 g/cm^3 per decade and in men 0.005×10^3 g/cm^3 and was more marked in the obese individuals. The gradient for the regression lines was less for the suprailiac than for the other 3 SkF, then the subjects of this study deposited more fat in this region compared to other sites in the body.

The amount of fat situated subcutaneously is a matter of controversy. There appears to be a common agreement that there is a linear relationship between subcutaneous and total fat mass and it is usually pointed out that the major part of store tissue is situated externally; however, there is a considerable variation in the reported proportion of fat or adipose tissue situated subcutaneously. Table 3.1 shows some of the studies that deal with

this topic, most of which have been referred by Durnin & Womersley, 1974 and Brown & Jones, 1977. The reported range of proportion of fat situated subcutaneously varies from 10 to 70%.

Brown & Jones, 1977 evaluated and compared their study to others to explain some of the possible errors and give some reasons for the differing found estimates. Compared to the study of Chien et al, 1975, they concluded that differences were due to the number and selection of SkFs. Neither Allen et al, 1956 nor Chien et al, 1975 corrected for compressibility; this fact alone could have accounted for underestimations of up to 25% in the estimated value. Another cause, is the constant factor used in the two studies to correct the dermis thickness. For their own study Brown and Jones admit that the proportion of fat in adipose tissue might differ between subjects with different degree of fatness and they used a constant value for all subjects. The low estimates obtained by Skerlj et al, 1953, may be explained because of the low factor used (0.42) for the amount of fat in adipose tissue.

Derivation of equations. The SkF method must be related to another method that can yield total body fat such as densitometry (D), the most widely employed method, or other methods which measure a body component such as potassium (K) or water (TBW), whose relation to FFM is fairly well established for selected groups.

The ability of SkF thickness to predict body density (B.D.) have been discussed in the reviews made by Coward et al, 1988 and Lohman, 1981 who calculated that the total variation (expressed as the standard error of estimate) in the relation of SkF to B.D. is 0.0098 g/cm^3 within a given population and 0.007 g/cm^3 in young men.

There exist a number of equations developed to predict B.D. or body fat from SkF and other anthropometric measurements (Brozek & Keys, 1951; Pascale et al, 1956; Durnin & Rahaman, 1967; Wilmore & Behnke, 1968, 1969; Durnin and Womersley, 1974; Jackson & Pollok, 1976, 1977; Sinning 1978; Withers et al, 1987 [a,b,c]; Jones & Satwanti, 1982;...). Lohman, 1981, reported that in various populations ranging from athletic to sedentary and from children to the aged, well over 100 equations using SkF alone or combined with other anthropometric dimensions have been developed in the last 30 years.

The equation to be selected, must be in accordance with the population to be evaluated. The equations more widely used worldwide because of the magnitude and variety of the population in terms of sex, age and physical characteristics, are probably those developed by Durnin and Womersley, 1974. Lohman, 1981 in a review of the studies relating SkF to body fatness as estimated from B.D.

stipulates that the estimation of B. C. proposed by Durnin & Womersley, 1974 is one of the more successful general approaches.

Durnin and Womersley, 1974 studied 209 men and 272 women between the ages of 16 and 72 years, healthy, preponderantly sedentary, middle class, deliberately selected to represent a variety of body types, British. They measured SkF thickness at 4 sites: biceps, triceps, subscapular and suprailiac and B.D. by UWW and the volume of air in the lungs at the moment of the measurement. Subjects were classified in age groups. Linear regression equations were formulated to estimate B.D. from the logarithmic transformation of single SkF and from the sum of two and more SkF for five age groups and each sex.

Author, year	Study	Results % SITUATED S.C.
Vierordt, 1906		≈ 50 % of F
Edwards, 1950	138 women SkF at 53 sites	% F increases as fatness does
Pochin, 1950	43 women	≈ 70 % of AT
Skerlj, Brozek & Hunt, 1953	84 women D & SkF at 10 sites	22 to 26 % F for different age ranges
Allen et al, 1956	87 Formosan women and men. D & SkF at diff. sites	20 to 60 % AT depending on total AT; lean 25-33%; obese 50%
Skerlj et al, 1953	3 groups of women of diff. age: 18-30; 31-45; 46-67 yrs.	26 % F first two gps. and 22 % third group
Chen, 1953 and Young et al, 1963	women of different ages	% F constant up to 45-50 yrs., then it decreases
Forbes, 1962	one neonate	42 % AT
Forbes & Amirhakimi, 1970	293 boys & 179 girls 8-18 yrs ^{40}K and SkF	% F higher for males than for females
Chien et al, 1975	Formosan subjects. D & SkF at diff. sites	50 to 60 % AT for AT between 15-40 kg
Brown & Jones, 1977	42 women 19-24 yrs. SkF at 11 sites	41-87 % F. Mean 65 ± 11
Alexander, 1964	20 cadavers	10 % F in women & 20 % F in men
Moore et al, 1968	cadaver of an elderly woman	32 % AT
Clarys et al, 1987	13 cadavers	The correlation between SkF with SC and total AT was high but with internal AT was non-significant indicating a dominance of SC AT
Pitts, 1956	72 guinea pig carcasses	22 % F in females and 16% in males. Constant for all degrees of fatness
Pitts & Bullard, 1968	32 non primate mammalian species	from 4 to 43 % F

Table 3.1. Variation in the proportion of fat (F) or adipose tissue (AT) situated subcutaneously (SC).

These authors found that the relationship between SkF and B.D. was not linear. In the more obese subjects relatively large increments in SkF were associated with only small changes in B.D.; they deduced then that the relationship was logarithmic or quadratic to yield a linear relation.

The wide variety of information about the distribution of fat in the body, makes it difficult for a general equation to be developed. In order to use an equation, an idea of the relation ext:int fat of the group to be measured, is necessary.

Lohman, 1981 has calculated a total error (biological plus technical) of 3.3% fat for a specific population but values as high as 5% fat were found by Durnin & Womersley, 1974.

9. ANTHROPOMETRIC MEASUREMENTS.

9.1. Body mass and Height: Body Mass Indexes.

Measurements of B.M. and height (H) do not actually provide information on B.C.. However, they are included here because B.M. related to H have long been used as an useful tool to classify subjects as normal, under or overweight; B.M. indexes are based on the ratios between B.M. and H to give an idea of B.C.

The underlying assumption in using a weight corrected for height index is that B.M., after correction for H, is highly correlated with a direct measurement of obesity. In the absence of a more direct measure of B.C. for comparison, the index should be consistently highly correlated with B.M. and independent of H. Lee et al, 1981 have examined the relative merits of these indexes and have concluded that from 4 of the traditional indices (W/H, W/H^2, W/H^3, and $H/W^{1/3}$) only W/H and W/H^2 were highly related to B.M. but all four were significantly correlated with H in various sex, and racial groups and thus their use as indices of obesity is not adequate in comparison studies. The issue, however, is not the degree of dissociation of H but the validity of the B.M. indexes to assess fatness in populations and individuals (Ross et al, 1988).

A problem with the use of these indexes is that big mistakes can be made as the one reported by Behnke, 1942 in which professional American football players were not accepted in the army for being overweight. 'No index which incorporates measurements of B.M. and H alone can differentiate between overweight caused by an excess of muscle and bone, and overweight caused by fat excess (Womersley & Durnin, 1977).

The body mass index (BMI), developed by Quetelet in 1836, is calculated as the B.M. [kg] divided by H [m] squared, and is probably the most common and widely used index, either for description of the

population studied, besides or instead of just using B.M. and H, and also to classify subjects as normal, overweight or obese (Garrow, 1981). The use of the BMI as a measure of body fatness has been found to give confusing results in general populations (Smalley, et al, 1990 and MacDonald, 1986). Durnin et al, 1985 compared relative fatness amongst large groups of individuals of the same H and B.M.; their conclusion was that the BMI and similar ratios could result in considerable error in the assessment of fatness. It would be obvious to expect even more confusion or erroneous results for subjects with unusual B.C. or for the elderly. Deurenberg et al, 1989 found that BMI in the elderly give somehow low values as compared to the body fat assessed by densitometry; because of the usual changes in B.C. with aging the same B.M. in an older subject means a completely different B.C. from that of a young adult.

The use of B.M. indexes as indicators of body fatness have shown to have wide disaffection; they have been proposed to be abandoned (McLaren, 1987), used with caution (Smalley et al, 1990), modified (Lee et al, 1981) or completed with other anthropometric data in order to adequately describe the biological characteristics of human populations or tests hypotheses regarding the relations between nutrition, growth, body size and disease (Micozzi & Albanes, 1987 and MacDonald, 1986). Garn et al, 1987 have explained that there are situations where B.M. and H are the only measurements taken and that while there is convenience in using these measurements alone in nutritional epidemiology, there is a finite limit to what can be accomplished with B.M. and H alone entailing a loss of crucial information bearing on morbidity, mortality and reproductive efficiency as well.

As a general consideration it can be said that B.M. and H are measurements that can be performed accurately, quickly, cheaply, and require minimal observer training. For these reasons almost all investigations include these measurements. However, if information on B.C. is required, additional measurements will be desirable, because of the limitations discussed above.

9.2. Body Girths and Bone Breadths.

Height on its own is an index of the skeleton size and if also bone breadths are measured they give an idea of bony frame-work.

Body girths can be used either to estimate fat and as indexes of muscularity in non-overweight groups of individuals. Being muscularity such a difficult variable to measure, comparison of groups can be done based on limb circumferences, although care should be taken because of the racial differences in the dimension of some girths, specially those of buttocks and thigh.

JUSTIFICATION AND OBJECTIVES.

Biological variations on the density of the fat free mass (FFM), on the amount of water and potassium (K) of the FFM, on the distribution of body fat, on the relationship of internal to external fat between subjects of different sex, age, physical type and physical activity, make wonder how appropriate are the assumed values attributed to different methods to estimate body fat and FFM on populations with characteristics that differ from each other.

The equations of Durnin & Womersley, 1974 have been shown to be of great acceptance for the assessment of body fat in subjects of different ages and physiques; however, the authors themselves admitted that at the lean end of the scale more information was needed. They found that there were too large variations in body fat for too small variations in skinfolds (SkF) and a very low value of the sum of four SkF (Σ4SkF) was still equivalent to apparently having moderate quantities of fat. For example, in the table of fat content as a proportion of B.M. a value of Σ4SkF of 20 mm corresponds to 14 and 8% fat for females and males, respectively, in the age group 17-29 years.

The present thesis shall examine the applicability of assumptions of constant density of the body components, constant amount of water and potassium of the FFM, constant body fat distribution, when applied to in groups of young adult and adult subjects of both sexes, at the lean and lean-muscular extreme of body composition. In order to include subjects with these characteristics, most of the subjects included in this study performed physical activities that were, on average, more intense than those performed by the general population, although there were some subjects that were included for their lean physical appearance although they were basically sedentary, owing their leanness probably to genetics.

Measurements included different entities of the body. i.e., total body density, total body water and total body potassium. With these three measures it shall be possible to examine some of the basic assumptions employed to estimate B.C.

With the use of the SkF method to estimate body fat using the equations derived by Durnin and Womersley, 1974, it will be possible to compare with other B.C. methods whether the results are comparable and whether the existing equations predict B.C. with fair agreement or whether it is necessary to develop special equations for the lean

group. Also, it shall help to review some of the assumptions that this method employs.

The aims of the present investigation were:

1.- to analyse the extent to which the results of body composition assessed by different methods compare, using for each method their 'classic or established' assumption(s) considered for the general population, on a 'more than usual' active, fairly lean and lean-muscular, young-mature population.

2.- to discuss on the application of the assumptions in this specific population, and

3.- when applicable and/or possible, to give a more appropriate value of density, K or water of the FFM, suggestions or new equations derived from SkF and anthropometry variables, to more accurately estimate body composition for subjects with the characteristics of the present investigation.

METHODS.

1. SUBJECTS.

There were 157 subjects that participated in this study, the recruiting and criteria for acceptance were described in the methods section of the BMR chapter.

2. TECHNIQUES USED IN THIS STUDY FOR THE ESTIMATION OF BODY COMPOSITION.

2.1. Measurement of Body Density by Underwater Weighing.

The determination of body density (B.D.) using the underwater (UWW) method, requires three measurements: body mass in air (BMa), body mass in water (BMw) while totally submerged (to find the mass of water displaced by the body) and then to determine the amount of air in the body at the time the measurement of the UWW is carried out. All the required procedure to take these measurements was carried out by the technician of the laboratory, and by the author of this work.

The procedure followed in this study for hydrostatic weighing was that described by Durnin & Rahaman, 1967 and Durnin & Womersley, 1974.

The equipment used, which has also been described by the above mentioned authors, was:

- Avery beam balance (W & T Avery Ltd. Avery House, Clerkenwell, London EC1. Model no. 3302)
- Tank. The apparatus used is shown in figure 3.4.. The dimension of the tank is 1.19 m x 1.19 m and 1.38 m depth; it has a capacity to hold up to 1900 litres of water, maintained at 36.5°C by means of a thermostatically controlled circulator. A canvas seat on a metal frame is suspended in the tank by means of nylon cords. This seat is, in turn, connected by hanging nylon strings to an instrument, suspended from the roof, for measuring the force exerted by the subject weight (Western Load Cell Co. Ltd. Scotland) and producing a signal diverted to a digital display unit, calibrated to read the mass in kg. Steps and supports were fitted to the outside and inside of the tank, to facilitate entry and exit.
- Anaesthetic bags (4 litres)
- 2-way taps (the third opening was sealed off with a rubber stopper)
- Mouth piece
- Nose clip
- Pure oxygen (about 99.2 %)
- Spirometer (Benedict - Roth type) with a capacity of 6 liters.
- A paramagnetic oxygen analyser (Servomex type 570 SYBRON, Servomex Ltd., Crowborough, Sussex, England)

- Infrared carbon dioxide analyser (PK Morgan Ltd. Chatham, Kent, England)
- a BBC micro-computer (model B) to speed calculations of body density and fat proportion.

Procedure of Measurements. Subjects were asked to come to the laboratory early in the morning without having eaten breakfast and on arrival were asked to empty their bladder. It has been demonstrated by Durnin and Satwanti, 1982, that a light breakfast would not alter the results to any considerable extent; however, as basal metabolic rate (BMR) was also being measured on the same day, subjects were asked to be in the fasting state.

- **Body mass.** Subjects were weighed outside the water wearing a skin-tight bathing suit. Readings were taken to the nearest 0.1 kg using a calibrated Avery beam balance.

- **Underwater weighing.** Subjects were guided to the room where the tank is located and asked to climb into the tank, sit down centrally on the chair, hold on to the sides of the chair with their hands and rest their feet on the cross bar placed below the seat to ensure that they did not come into contact with the floor during the measurement.

The level of the water, previously heated to a temperature of about 36°C, was adjusted to cover up to the neck of the subject, below the chin; her/his head was just above water (figure 3.5).

Subjects were asked to get their hair soaked in order to express all the bubbles, the whole procedure was explained to them, a nose-clip was fitted; they were asked to bend slowly forward from the waist and to gradually submerge completely, without touching the walls of the tank; then they practiced it as many times as necessary (usually twice) to familiarize with the method and be sure that they had fully understood it.

Determination of the residual volume. The determination of the residual volume (RV) was carried on using the nitrogen wash-out method described by Rahn et al, 1949 and modified by Durnin & Rahaman, 1967 and Durnin & Womersley, 1974. Only the volume of air in the lungs and respiratory passages were measured.

The trials for the RV were done asking the subject to give a total expiration before bending forwards and when she/he was asked to get his head out, to hold her/his breath and using the mouth piece to breath deeply in and out using a dummy 2-way tap with no bag attached.

This measurement was done immediately after the body mass underwater (UWW) had been recorded. Anaesthetic bags were previously

filled with a known volume of oxygen. The bags must first be washed out with pure oxygen and completely evacuated with a vacuum pump before final filling with a known volume (about 3 liters) of pure oxygen from the spirometer (figure 3.6).

The temperatures of: the water in the tank, the spirometer and the air were measured and the atmospheric pressure was read, for every measurement, to calculate the correction factor (see below).

Indications given to the subjects. Once the subjects appeared to be confident and relaxed, they were asked to follow the protocol to measure their BMw and their Res. vol. The specific indications were given as follows:

- make a maximal expiration,
- with your mouth firmly closed and holding your breath, bent as gently as possible forwards until your head is completely immersed just beneath the water surface,
- maintain your position, keep as still as possible, stay very quiet. (This position was maintained until the digital display stabilized enough to take the reading of the BMw. This procedure usually lasted between 10 to 15 seconds) (Figure 3.7).
- when you hear the signal (a boom on the side of the tank) you may surface gently. Hold your breath...

For the determination of the **residual volume** (figure 3.8), the above indications were immediately continued.

- ... hold your breath until your lips are tightly over the mouth piece (a mouth piece attached to one limb of a two way tap, was placed in between the lips of the subject; the other end of the tap was connected to a rubber anaesthetic bag containing a known volume of pure oxygen; the tap was opened),
- take a deep inspiration from the bag and continue breathing in and out the bag 3 times (at the end of the third cycle of inspiration-expiration, the tap was closed),
- release the mouth-piece.

When the measurement was over, the bag was taken away; the mixed air -with the N in the lungs and the oxygen of the bag- was immediately analysed for the O_2 and CO_2 and N content determined by difference. The whole procedure was repeated twice; were there not a good agreement between the 3 measurements a fourth, or even a fifth, measurement was taken. A fat% disagreement between measurements of equal or greater than 3% of B.M. was the criteria used to perform another measurement. This was possible to do because calculations of Res. vol., B.D. and body fat% were done using a microcomputer while the subject was still in the tank, allowing to

calculate the results fast enough. The mean of the three calculated results for density was taken as the final value for the subject.

An interval of about 5 minutes was allowed between each measurement to allow the N concentration of the lungs to return to normal values.

Calculations.

a. Residual Volume (Res.vol.). The sequential steps to achieve this equation were expounded in the literature review on densitometry, section 5.2.

$$Res.vol.[L] = \frac{N(V+0.025) - 2 - Vn}{80 - N} \times F$$

where:

N	=	N_2 proportion in the final sample (100-(O_2 + CO_2)
V	=	volume of pure oxygen of the bladder [L]
0.025	=	25 ml of dead space in the 3-way tap divided by 1000
80	=	alveolar N_2 concentration in the residual air (80%).
2	=	product of multiplying (80 * 25) divided by 1000.
Vn	=	initial volume of N_2 in bladder (≈0.8 * V)
F	=	BTPS is the barometric pressure and temperature correction factor, and is calculated as follows:

$$F = \frac{273+37}{273+t} \times \frac{A.P. - p}{A.P. - 47.1}$$

where:

273 = absolute T°; **37** = assumed body T° in °C;

t = T° in the spirometer in °C;

A.P. = atmospheric pressure [mm Hg];

p = partial pressure of water vapour at spirometer T° [mm Hg] got from Saturation Pressure of water vapour table;

47.1 = partial pressure of water vapour at body T° [mm Hg].

b. Body density (B.D.).

It was calculated using the equation:

$$B.D.[g/cm^3] = \frac{BMa}{\frac{BMa-BMw}{Dw} - Res.vol.} \times 100$$

where:

BMa and BMw = body mass in air and in water, respectively
Dw = density of water at the water temperature in the tank.

The proportion of body fat was calculated using Siri's equation (1956), see literature review on densitometry, section 5.

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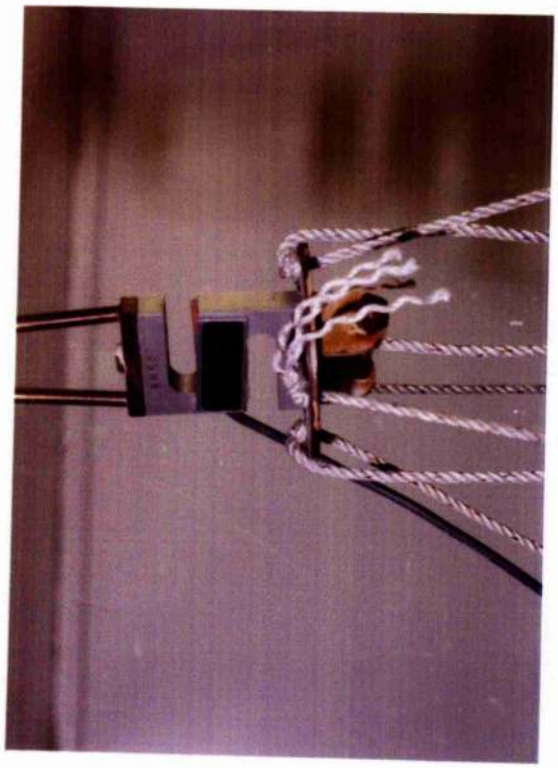
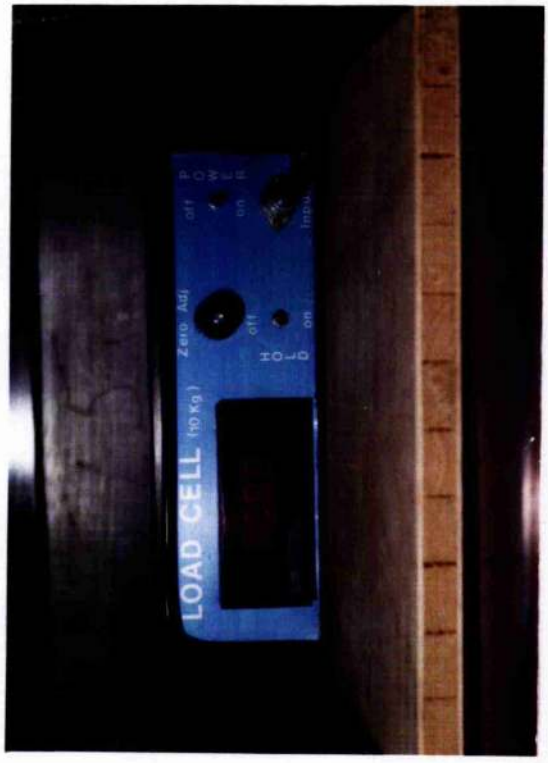




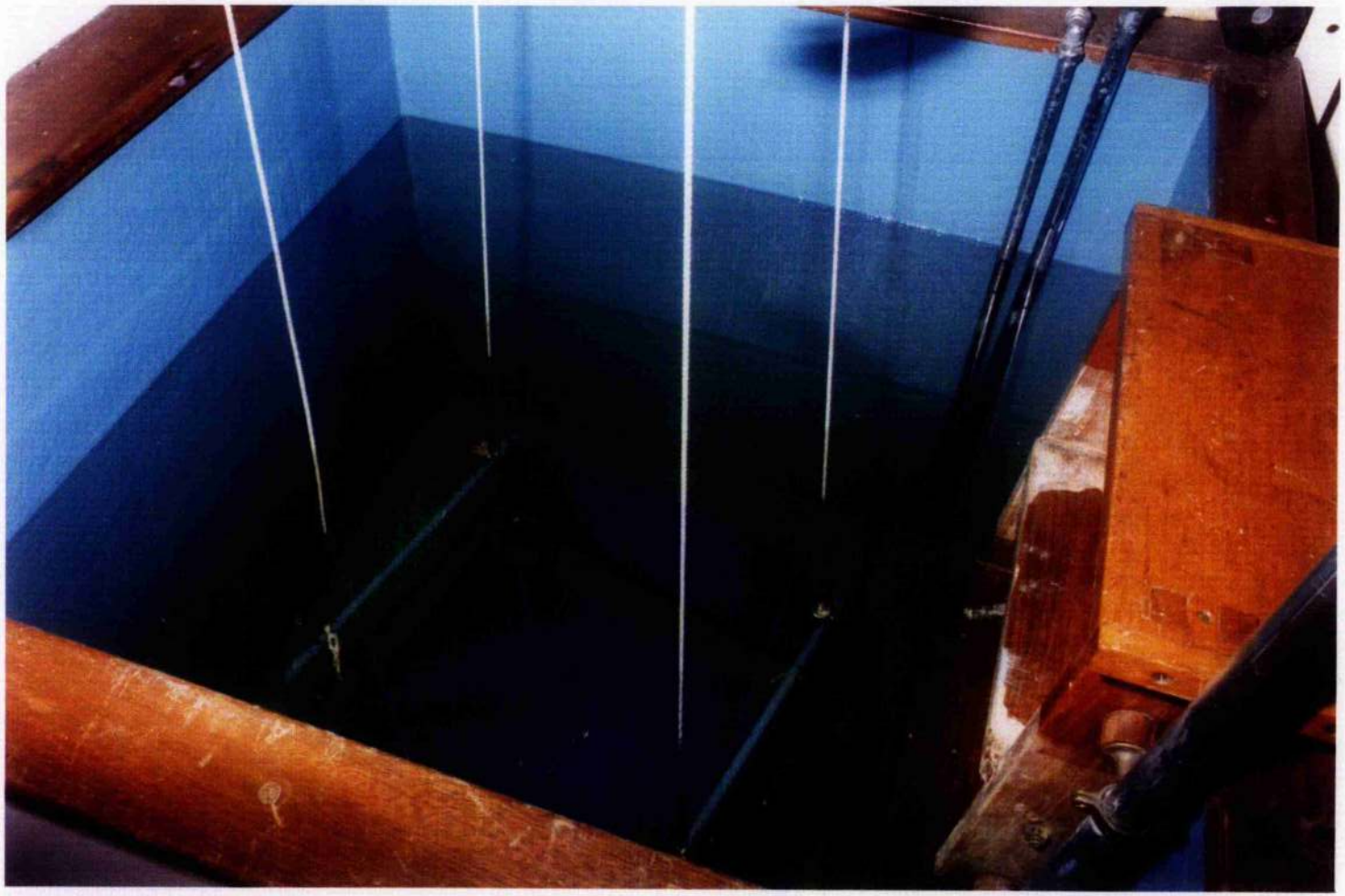


Figure 3.4. Apparatus to measure body density by underwater weighing.

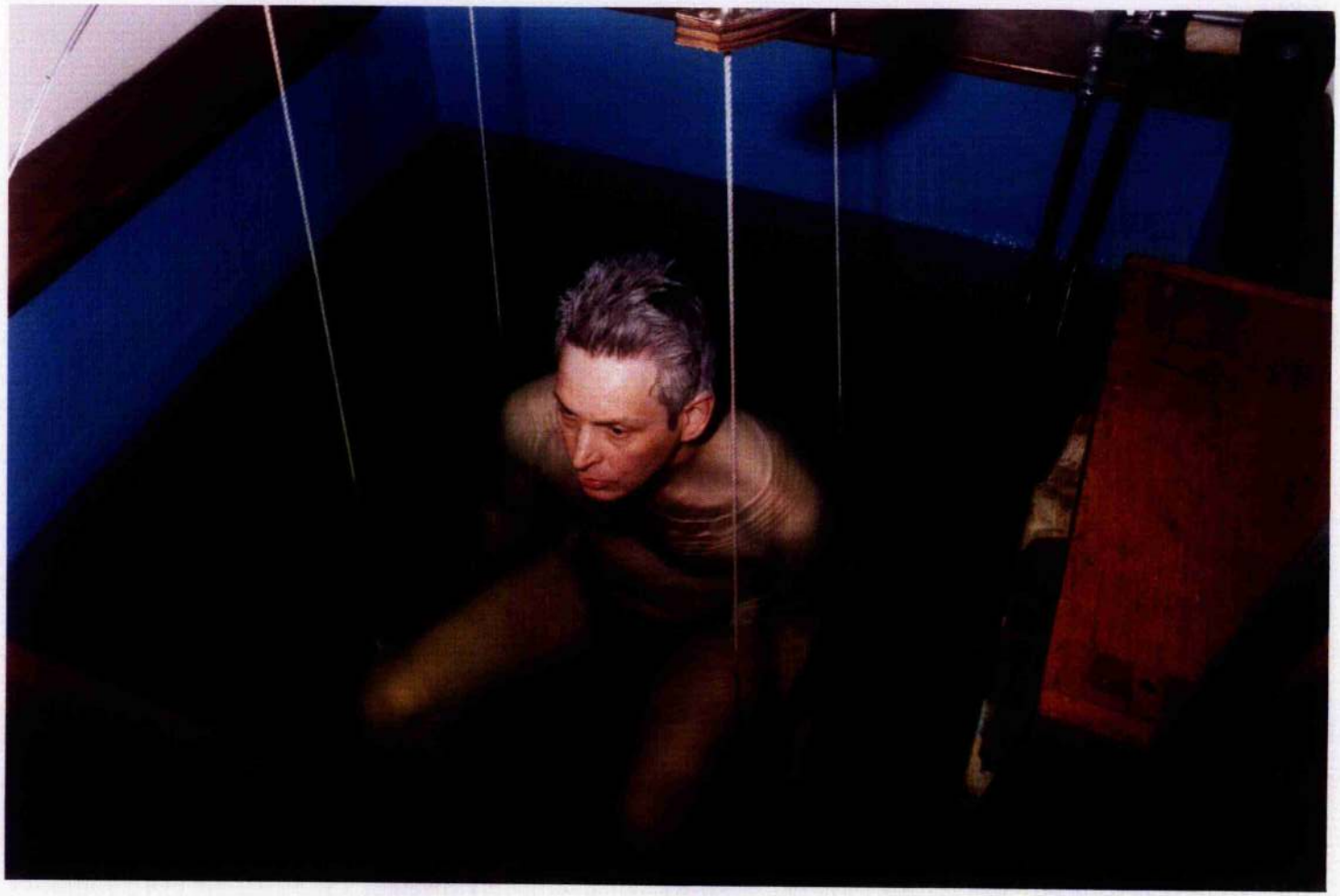


Figure 3.5. Measurement of body density by underwater weighing. The subject is shown sitting on the weighing chair, his head just above the level of the water.

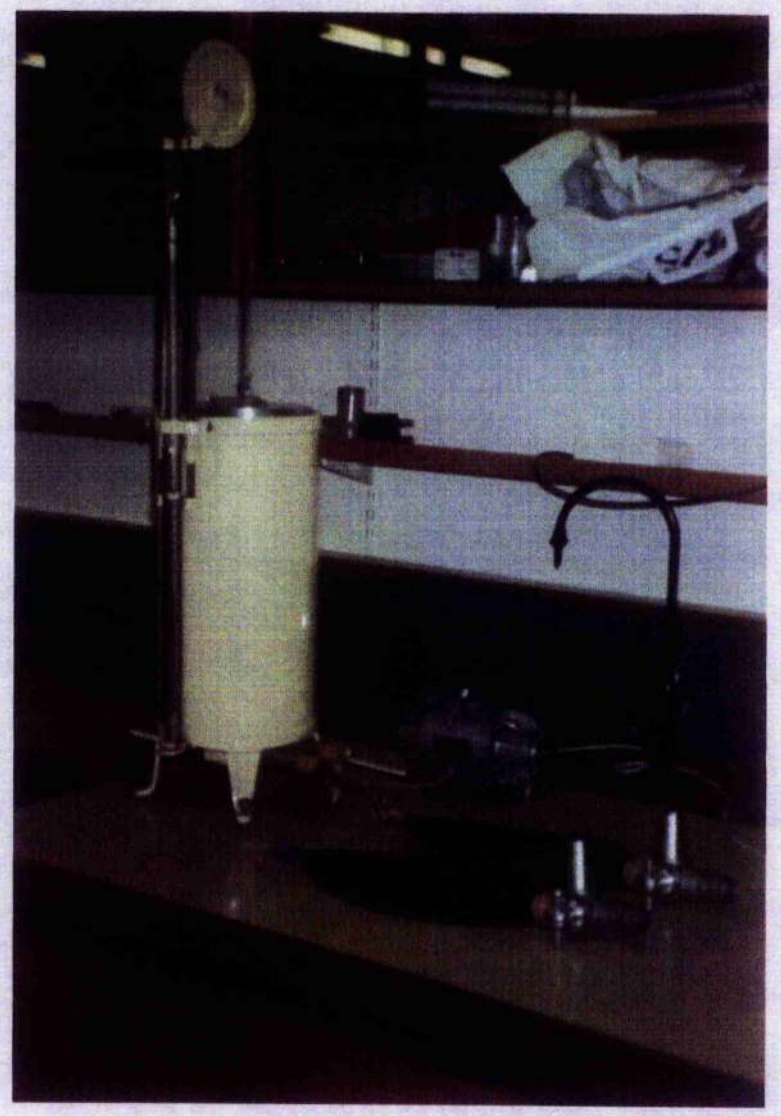


Figure 3.6. Equipment used for filling the rubber bags with oxygen.

The picture shows the spirometer, which was first filled with oxygen from the cylinder.
It can also be seen the 4-litre anaesthetic bags and the vacuum pump with which the bags were evacuated before final filling.

Figure 3.7. Measurement of body density by underwater weighing. The subject is shown with his whole body immersed in water.

Figure 3.8. Measurement of the residual volume.
The subject is shown with the mouth piece in between his lips at the time of the re-breathing cycle.

2.2. Total body water (TBW).

In this study, because of the availability of the equipment, deuterium oxide (D_2O or (^2H_2O) (Sigma Chemical Co., Poole, Dorset, England) was the selected as the tracer and mass spectrometry was the analytical technique. This is usually the procedure to use because measurements of the order of less than 1% precision on dilution values can be achieved. The procedure is based on the description made by Halliday et al, 1977 and modified by M. Lawrence, 1990 at Durnin's laboratory, which is as follows:

The tracer will mix throughout the total body water pool. The subsequent concentration in a sample of body water (saliva, urine or plasma) once equilibrium has been achieved provides an estimate of the TBW. The dose of the isotope given to the subjects must be large enough to produce a readily measurable increase in the D_2O concentration (C) of the body. If

$$C = \frac{D}{TBW}$$

and

$$TBW = \frac{D}{C}$$

where:
C = concentration of the tracer and
D = dose given

then, C must be large in relation to the precision with which it can be measured. It was estimated by Lawrence, 1990 that for the analytical error to be < ± 1%, an increase in D_2O concentration of at least 180 ppm was necessary; this is equivalent to a dose of 0.1 g D_2O per kg of TBW. In practice, to allow for a slight margin of error, a dose of 0.12 g of D_2O /kg TBW was used. Doses were given per kg body water since it is the compartment that is to be labelled.

A previous estimation of body water was made from the body mass and the FFM obtained from skinfolds and assuming that TBW occupies a fixed fraction of the FFM of 73%.

Equipment.

a. for preparing and administering the dose: a balance accurate to 0.01 g; 20 ml plastic bottles; drinking plastic straws.

b. to collect saliva samples: cotton wool; small sticks (to wrap cotton around); plastic tweezers; 2 ml disposable syringes; wire (to remove cotton wool from syringe after taking sample).
c. for the storage of samples. freezer; 1.5 ml sample tubes (labelled with subject's number and sample code).

Detailed Procedure.

a. Preparation of dose. In advance of the study a 40% solution of D_2O (99.8%) had been made up (two 1 ml samples of this diluted solution were kept for further analysis). This solution was then used to prepare individual doses corresponding to a given estimated TBW, i.e., 0.3 g of the 40% solution per kg of estimated TBW had to be weighed. Each dose was kept in a 20 ml plastic bottle, sealed with sleek tape and refrigerated (to prevent bacterial growth) until required.

Example: For a subject weighing 65 kg, with an estimated FFM of 57.2 kg, his estimated TBW would be (57.2*0.73) = 41.8 kg. The dose to give to this particular subject would be (his estimated TBW * dose of D_2O/kg TBW [g/kg] (41.8*0.12)) 5.01 g of D_2O. Since from a practical point of view it is easier to weight and drink a larger volume than this, using the 40% diluted solution the dose given to this subject would be (41.8*0.3) 12.5 g.

The procedure of preparing series of doses as just described, was necessary because D_2O is hygroscopic and as such tends to absorb water vapour from the air. Repeated opening of the stock solution to make up individual doses on a daily basis would have slightly changed the composition of the solution during the course of the study. Then, series of doses were prepared based on the most probable FFM ranges expected to be found. Once body mass and SkF measurements were performed and TBW estimated, the most appropriate individual diluted dose of deuterium was selected and administered to the subject.

b. Subjects. Subjects arrived to the laboratory early in the morning without having exercised or consumed any alcohol the previous day (to control hydration) and in a fasting state (of about 12 hours). They were asked to empty their bladder, weighed (wearing only a light gown of known weight and underwear) and were not allowed to drink nor to eat anything throughout the duration of the study (3 hours). The anthropometry measurements that were taken have been described in the literature review on anthropometry, section 4.

c. Administration of the deuterium oxide and saliva samples. As deuterium is naturally present in body water it was necessary to establish the background level of the isotope concentration before

any additional dose was given. This was done by taking a saliva sample previous to the administration of the D_2O dose.

Once the adequate dose was selected for the subjects, then:

- the isotope dose in its closed bottle and a plastic straw were weighed on a balance accurate to 0.01 g.
- the bottle was opened immediately before the dose was administered (to avoid evaporation). The subject then drank as much of the isotope as possible, using the plastic drinking straw and taking care that none was spilled.
- the straw was pushed down inside the bottle and the lid put back on. The whole thing was then reweighed to know how much of the isotope was drunk by the subject.
- the calculation of the exact diluted isotope drunk by the subject was done, from which, according to the dilution factor and the percentage of deuterium in the dosing solution, the amount of D_2O ingested could be computed.

After the administration of the dose, saliva samples were collected two and three hours after the administration of the dose using the sample procedure described below. Complete equilibration of the deuterium with the body water pool should have been achieved after about two to three hours. The concentration of D_2O at plateau however, can be taken as the mean of the two samples. This approach to some extent allows for any random fluctuations in D_2O concentration at equilibrium and further, it provides confirmation that the plateau stage had in fact been reached.

Collection of samples. Throughout the investigation saliva was taken as the representative body fluid because, as previously explained, a post-dose plateau is achieved more rapidly than with urine.

The saliva was collected by wrapping a small piece of cotton wool around a stick and asking the subject to move it around his/her mouth until it was 'soggy'. Care was taken that the sample was not full of mucous. The cotton wool was removed with a piece of wire and then transferred using tweezers to avoid contamination, to a 2 ml plastic syringe and the saliva was squeezed out into an appropriately labelled 1.5 ml sample tube. This procedure was repeated until sufficient saliva had been collected, ideally about 1-1.2 ml (the minimum required is 0.5 ml). Care was taken to not overfill the sample tubes because the top may be pushed off when the sample is frozen. The sample was then sealed and frozen at -20°C until analysis.

Sample tubes were labelled with the subject's number (S001-S150) and the sample code for the pre-dose sample (S_0) and the post-dose samples (S_2 and S_3) and sampling details such as date, time collected, etc. were recorded in a sampling form for each subject.

The syringes were rinsed out after use with clean water making sure that they were dried before they were used again.

Urine passed during the equilibration period. Urine passed during the equilibration period will contain some isotope. Some workers measure the volume of urine and take samples so as to adjust for this. This was not done in this study since under reasonable standardized conditions, losses of water and tracer during the equilibration period are likely to be of little practical significance and can be ignored (see lit. rev. section 6.1.).

Measurement of deuterium oxide in saliva. The deuterium oxide concentrations of the saliva samples are on the waiting list to be determined by isotope ratio mass spectrometry (Aqua Sira. VG Isogas, Chesire, UK) at the Scottish Research Universities and Reactor Center, East Kilbride.

The calculations that will be presented herein are those followed to estimate TBW at Durnin's laboratory; they were adapted by M. Lawrence, 1990 from those proposed by Coward, and Lawrence showed that both calculations were equivalent.

This measurement is based on the principle that the deuterium atoms in deuterium oxide (^2H_2O) have a different mass from those of normal water (H_2O). Consequently, mass spectrometry can elucidate the relative proportions of the two forms of hydrogen in a sample of fluid, in this case saliva. Water from the sample is reduced to hydrogen gas before measurement. Because relatively little deuterium is present in the samples (<500 ppm) there is little chance of any D_2 molecules forming and it is the ratio of DH/H_2 that is measured. It is then necessary to convert the isotope ratio (in ppm) into a concentration.

Calculations.

a. Conversion of isotope ratio into concentration:

Let **R** be the result in ppm obtained from the mass spectrometer.

$$R \times 10^{-6} = \frac{partsDH}{partsH_2}$$

and

$$0.5\times R\times 10^{-6}=\frac{partsD_2}{partsH_2}=\frac{partsD_2O}{partsH_2O}$$

For measurement of TBW R<600, ratios and concentrations can be considered equivalent, i.e., the concentration of D_2O in parts of D_2O/parts of water can be taken to be $0.5*R*10^{-6}$.

b. Conversion of parts to weights: molecular weight of H_2O = 18; molecular weight of D_2O = 20
therefore,

g D_2O = parts D_2O x 20
g H_2O = parts H_2O x 18

$$=\frac{0.5\times R\times 10^{-6}}{0.9}$$

and the concentration of D_2O in water [g/kg]

$$=\frac{R}{1800}$$

c. Calculation of TBW:

$$TBW=\frac{d}{C}$$

$$C=\frac{Rs-Rp}{1800}$$

where: p and s denote pre- and post-dose samples, respectively and

$$TBW=\frac{1800d}{Rs-Rp}$$

...equation 1

d. Calculation of dose: It is convenient to measure the deuterium oxide content of the dose solution at the same time as the samples

and on the same mass spectrometer; in this way, errors in the measurement of TBW are minimized. A sample of the dose ('a' [grams]) was diluted to a total mass of 'W' [g] with tap water.
From equation 1,

$$W=\frac{1800y}{Ra-Rt}$$

where:
'y' is the amount of D_2O in a grams of dose;
'Ra' is the concentration of D_2O in the diluted dose and
'Rt' is the concentration of D_2O in the tap water used to prepare the diluted dose.
Re-arranging,

$$y=W\frac{Ra-Rt}{1800}$$

and % D_2O in the dose

$$=100\times\frac{Y}{a}=\frac{W\times(Ra-Rt)}{a\times18}$$

...equation 2

If 'A' g dose are administered, then the weight of the D_2O administered

$$d=A\times\frac{W\times(Ra-Rt)}{a\times1800}$$

...equation 3

Analysis of samples and screening of the results. For a single determination of TBW a minimum of 4 analysis should be carried out. The pre-dose saliva sample should be measured in duplicate and each of the post-dose samples should be measured once only. For duplicate analysis of the same sample the maximum difference between samples should be 3 ppm. The mean and SD of duplicate analyses (1st-2nd) should be zero and <2 ppm, respectively.

Differences between 2nd and 3rd hour post-dose saliva samples should also be examined. Larger difference between these samples can

be expected than in the case of duplicate analysis of the same sample because of random fluctuations in D_2O concentration at plateau, but the maximum difference should be no greater than 8 ppm. The mean difference between samples should be zero (otherwise a plateau has not been reached) with a SD < 4 ppm.

In pre-dose samples R should be in the range 300-310 ppm and in post-dose samples R should be about 520 ppm.

Calculation of results.

From equation 1,

$$BodyFat = Wt - \frac{TBW}{0.73}$$

$$\%Bodyfat = 100 - \frac{TBW}{0.0073 \times Wt}$$

The use of D_2 as a tracer results in the over-estimation of TBW because some of the D_2 in the dose exchanges with labile H_2 atoms in cellular material mainly in carboxyl, hydroxyl, and other groups in which H_2 is not bound to carbon. The D_2 space, while identical to that of normal H_2 in water, is nonetheless greater than TBW by the same proportion in which exchange occurs. Overestimation of TBW has been estimated by Schloerb et al, 1950 to be about 2% of B.M. However, it has been suggested (Prentice et al, 1952) that the precise value depends upon the relative amount of lean tissue, and, hence, the error relative to B.M. will be greater in the leaner individual. Based on this, in the present study the value of the correction to be used has been estimated to be about 4%. Therefore,

$$True\text{-}TBW = \frac{Calculated\text{-}TBW}{1.04}$$

In the calculation of body fat [kg and %] the correction factor 1.04 was therefore applied, i.e., 0.73 * 1.04 = 0.759:

$$Body\text{-}fat[kg] = Body\text{-}mass - \frac{TBW}{0.759}$$

$$Body\text{-}Fat[\%] = 100 - \frac{TBW}{0.00759 \times Body\text{-}mass}$$

2.3. Total body potassium.

Procedure of measurement and apparatus. Measurements were made using a dual-detector shadow-shield whole body counting system of high sensitivity developed by Boddy et al, 1975. This apparatus is found at the Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow.

Subjects were transported from the University of Glasgow to this place; the author of this work always accompanied the subjects of the study but all the procedure was carried on by the expert technician of the Centre.

Measurements were done with the subject lying on a motorized couch, who passed in between the detectors and was scanned several times from feet to head and then from head to feet in the supine position, the constant speed of 5.2 cm/min was irrespective of the load carried on the couch (figure 3.9.).

The detectors (large crystal of sodium iodide), one situated in a central turret and the other one below in line with the first, are housed in a stainless steel casing and have 7 photomultipliers. The signals from the photomultipliers are combined and fed into a unity-gain preamplifier, which acts as an impedance matching unit. The analyser system consists of a computer connected to the detectors through two analogue-to-digital converters and two amplifiers. The associated peripherals comprise a teletype, a printer, a paper tape punch, a paper tape reader and an 'X Y' display. Commands are input to the analyser system from the teletype or from the high-speed paper tape reader (figure 3.7.).

For patient measurements, counting is controlled by two micro-switches fixed to the monitor which are operated by actuators mounted on the couch, allowing the scan length to be varied according to subject height within the range 140-200 cm. The micro-switches, through a micro-relay, cause pulses to be transmitted to the computer, which enables and disables the analogue-to-digital converters accordingly (Boddy et al, 1975).

Results. The subjects' counting rate in the potassium-40 photopeak (1.36-1.46 MeV) was expressed as mmol or g of potassium and was calculated using the regression equation described in the literature review section 7.1. A single whole-body measurement was

performed and the administration of a radioactive isotope (^{42}K) was avoided, using the calibration procedure described by Boddy et al, 1971, also described in the same section.

Calibration. Calibration of the instrument was performed before measurements on each group of 2-4 subjects, using counting phantoms composed of varying numbers of plastic bottles, containing known amounts of potassium, arranged in such a way to simulate the shape and proportions of the human body (figure 3.11.).

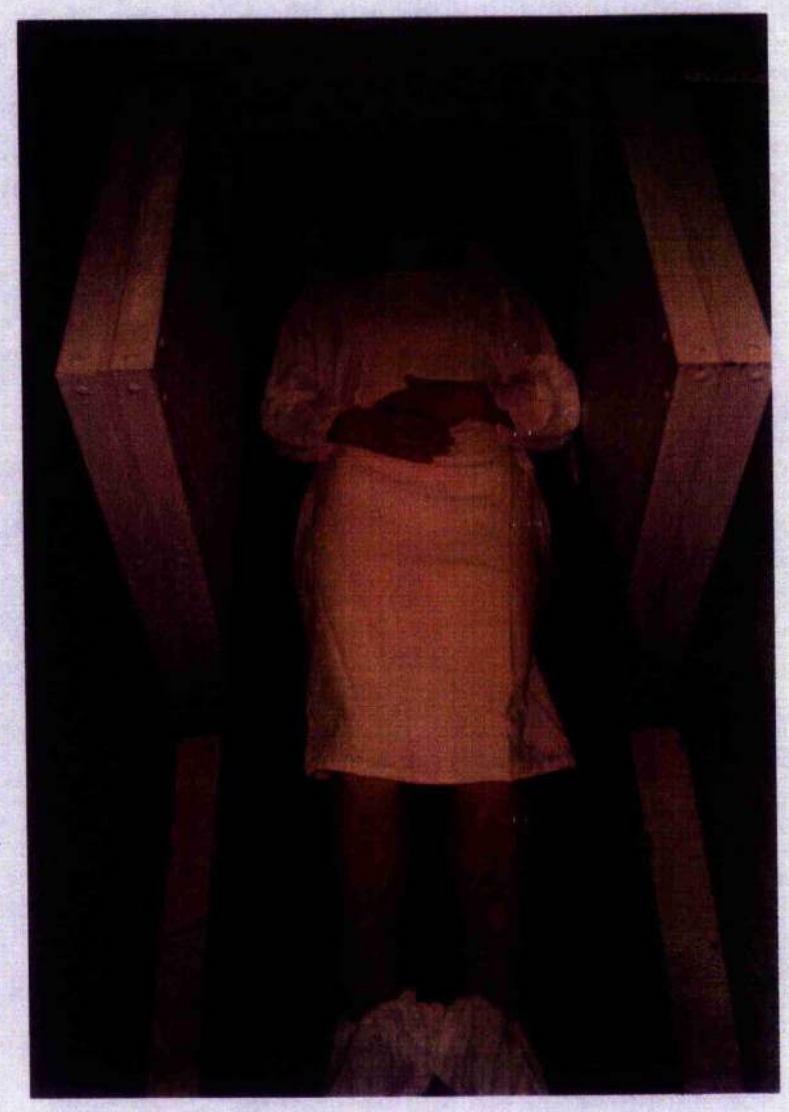


Figure 3.9. Measurement of total body potassium.

The subject is shown lying in the supine position on the motorized couch, wearing a gown, cap and slippers, coming out from the detectors, when she was being scanned from feet to head.

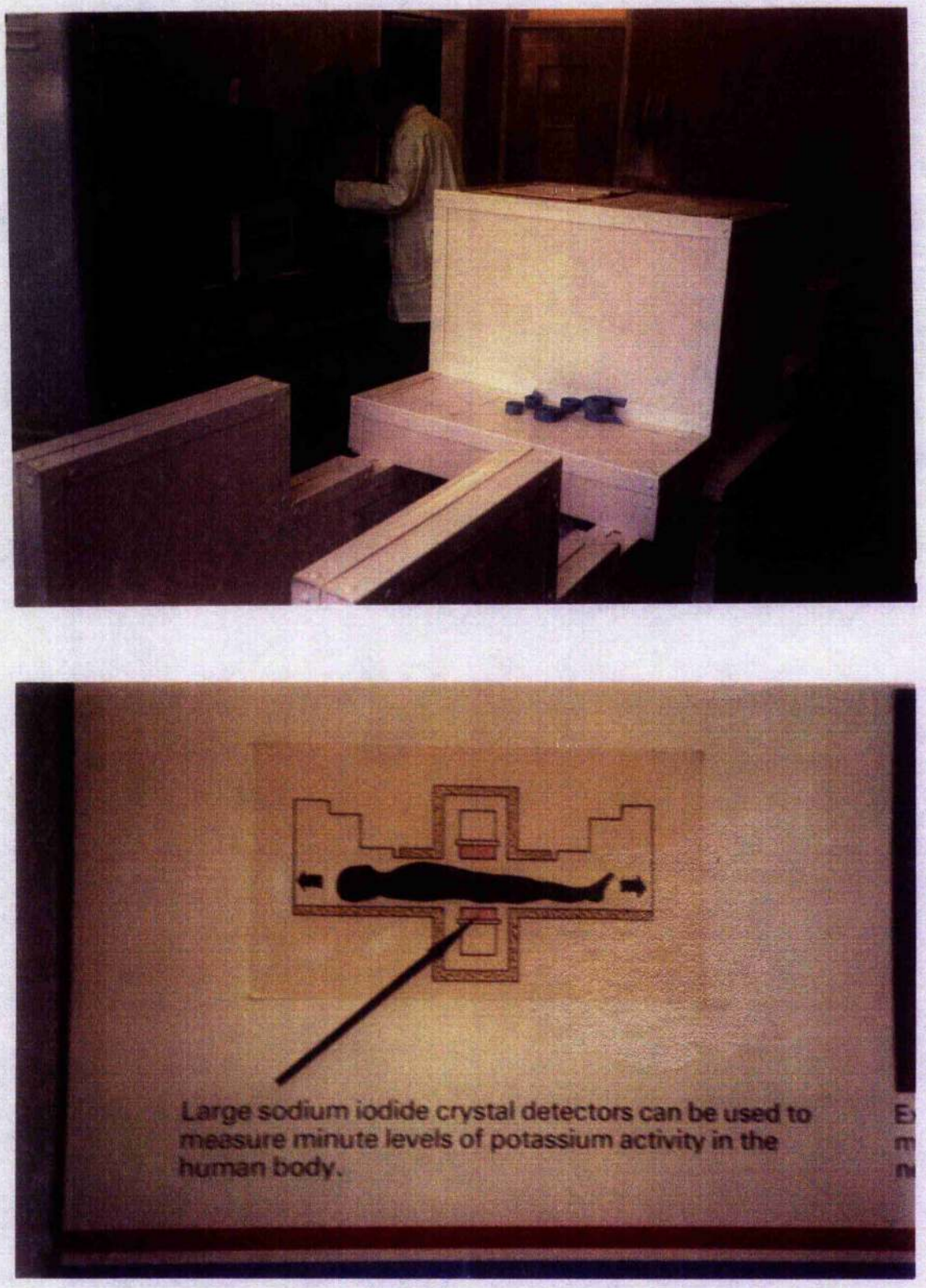


Figure 3.10. The whole body counter and the analyser system and diagram showing the counter and the position of the detectors.

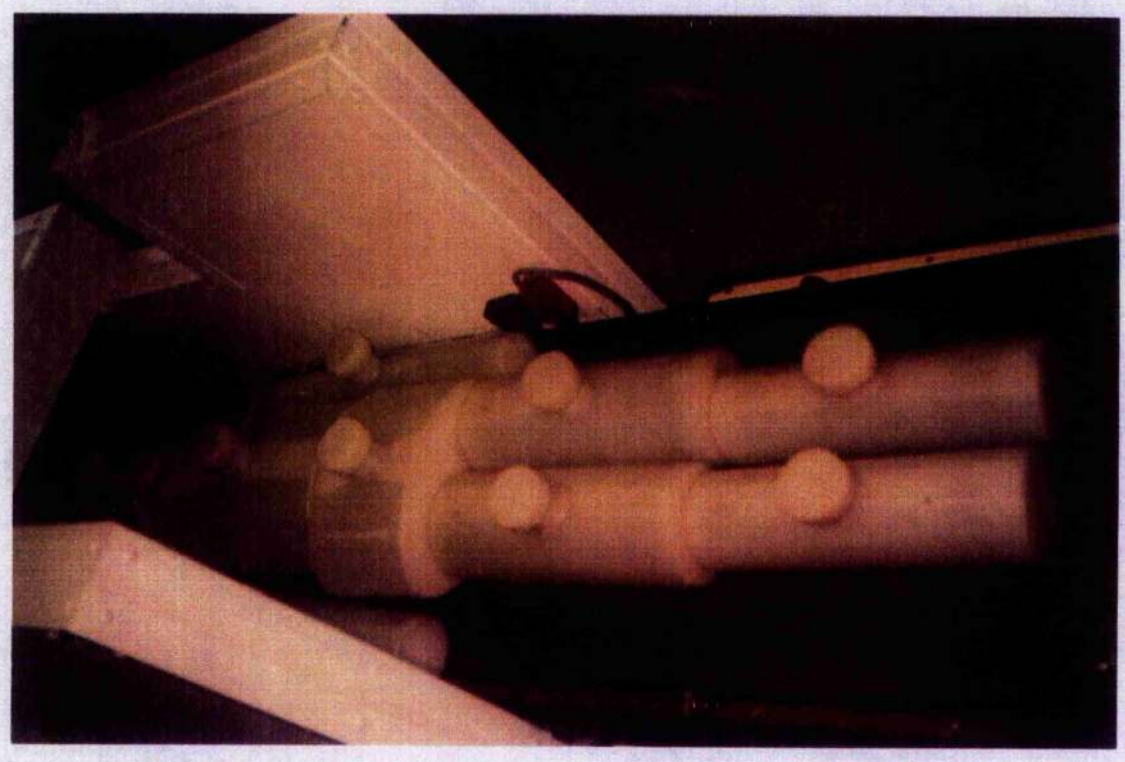


Figure 3.11. Calibration of the whole body counter with a phantom of known potassium content.

Nine bottles are situated in the motorized couch, to simulate the proportions of the human body.

2.4. Anthropometry.

The selected measurements for this study were those in use at the laboratory of Prof. JVGA Durnin who has suggested them as being appropriate for an anthropometric study, and have been considered to give assessment of frame size, muscle mass and body fat. All measurements were performed by the author of this work, in the morning and except where indicated as described by Weiner and Lourie, 1969 in the I.B.P. Handbook No.9, and are:

- body mass and height
- body girths: upper arm, waist, buttocks, thigh and calf
- bone breadths: biacromial diameter, bi-iliac diameter, wrist breath and knee
- skinfolds: biceps, triceps, suprailiac and subscapular.

Body Mass. Weighing was carried out with the subject clothed in bathing suit or in underwear and a light gown, after she/he had emptied their bladder. Readings were taken to the nearest 0.1 kg using a calibrated Avery beam balance (W & T Avery Ltd. Avery House, Clerkenwell, London EC1. Model no. 3302).

Height. Each subjects stood, without shoes, on the horizontal platform of the Wall Harpenden Stadiometer (Holtain Ltd. Grymych, Dyfed, England, UK), with heels together, well in contact with the floor (verifying that subjects would not rise them), and with their arms hanging naturally by their side.

Subjects were asked to stretch upwards to their fullest extent; for this they were asked to take a fullest breath and to blow out gently. They were also asked to hold their back as straight as possible against the vertical bar of the stadiometer and in line with head and heels. The "Frankfort plane" was checked to be correctly positioned: head erected, with the inferior outer edge of the eye in the same horizontal plane as the external auditive conduct.

Once ready, the head bar was brought down on to the head, pushing gently, and the readings were recorded to the nearest millimetre (mm).

Body Girths. All circumferences were taken with the subject standing in a relaxed position, except for the calf circumference which was taken with the subject seated, on the right side of the body. Measurements were made using a flexible steel tape (Harpenden Anthropometry Tape (2 m x 1 mm) Holtain Ltd. Grymych, Dyfed, UK). The tape was placed firmly around the position of measurement and the reading was recorded to the nearest mm.

Upper Arm: the subject's arm hung relaxed, just away from its side. A horizontal circumference was taken mid-way between the inferior border of the acromion process and the tip of the olecranon process.
Calf: the subject sat with their legs relaxed and their feet resting on the floor, with its knees bent to a 90° angle. The maximum horizontal circumference was located by moving the tape up and down and the measurement was performed.
Thigh: the subject stood with their feet slightly apart, its weight evenly distributed on both feet and relaxed. The measurement was taken with the tape placed around the thigh horizontally with its top edge just under the gluteal fold.
Buttocks: the maximum horizontal circumference over the buttocks was taken with the subject standing, relaxed and with its feet together.
Waist: the smallest horizontal circumference was measured, this point is usually found midway between the last rib margin and the iliac crest, at the level of the waist narrowing as seen from the front. The subject was asked to breathe gently, to prevent she/he from contracting its muscles, while this measurement was being taken.

Bone Breadths. Biacromial and bi-iliac diameters and right wrist and knee breaths were measured using a long arm anthropometer and a sliding caliper (Holtain Ltd. Grymych, Dyfed, UK), respectively. For all these measurements strong pressure was applied to compress the tissues overlying the bone. Measurements were recorded to the nearest mm.
Bistyleon or wrist breadth: the breadth was taken across the styloid processes (oblique to the long axis of the arm).
Bicondylar femur or knee breadth: the subject sat with its knees bent to a 90° angle, the breath was measured across the outermost parts of the lower end of the femur.
Biacromial: the subject stood with its shoulders relaxed and backwards to give maximum shoulder width. Standing behind the subject, the outside edges of the acromion processes were located and the breadth was measured placing the two arms of the anthropometer along the lateral borders of the acromion processes.
Bi-iliac: the subject stood with its feet together and the anthropometer arms were brought into contact with the iliac crests at the site which gave the maximum diameter. Standing behind the subject, strong pressure was applied to the anthropometer blades to push aside the fat covering the bone, and the measurement was taken.

Skinfolds. Skinfold (SkF) thicknesses were measured with the subject standing in a relaxed position, to the nearest 0.2 mm and on the right side of the body (although, Durnin & Womersley, 1974 found

non statistical difference between measurements on either side of the body), using Harpenden calipers (Holtain Ltd. Grymych, Dyfed, UK), calibrated to exert a constant pressure of 10 g/mm^2.

The SkF was picked up firmly between the thumb and forefinger, about 1 cm above the point (previously marked) where the measurement was to be measured, and pulled gently away from the underlying tissues; care was taken to ensure that no muscle was contained in the SkF and that subcutaneous adipose tissue as well as skin was present. The calipers were applied to the fold exactly at the sites described below (figure 3.12). The jaws of the caliper were released to exert its full pressure on the SkF, the finger and the thumb were removed and the reading was taken when the rapid decrement had ceased (about 2-3 seconds) and the pointer began to stabilize. Each SkF was measured and recorded in triplicate and the average value was used for the sum of the 4 SkF (Σ4SkF) thicknesses to estimate body density using the prediction equations of Durnin & Womersley, 1974. Lastly, body fat% was calculated using Siri's equation (1956).

Biceps: this SkF was measured vertically on the front of the arm directly above the centre of the cubital fossa at the level of the mid-point of the muscle belly while the arm was hanging vertically.

Triceps: a vertical SkF was taken after making a mark at the back of the arm, half way between the inferior border of the acromion process and in line with the point of the olecranon process, with the arm hanging vertically.

Subscapular: this SkF was picked up just below the tip of the scapula at an angle of about 45° to the vertical (modified by Durnin & Rahaman, 1967 and Durnin & Womersley, 1974).

Suprailiac: a vertical SkF was picked up in the mid-axillary line immediately above the iliac crest (modified by Durnin & Rahaman, 1967 and Durnin & Womersley, 1974). On the event that this were not possible to raise adequately, a perpendicular or horizontal SkF was measured at the same site.

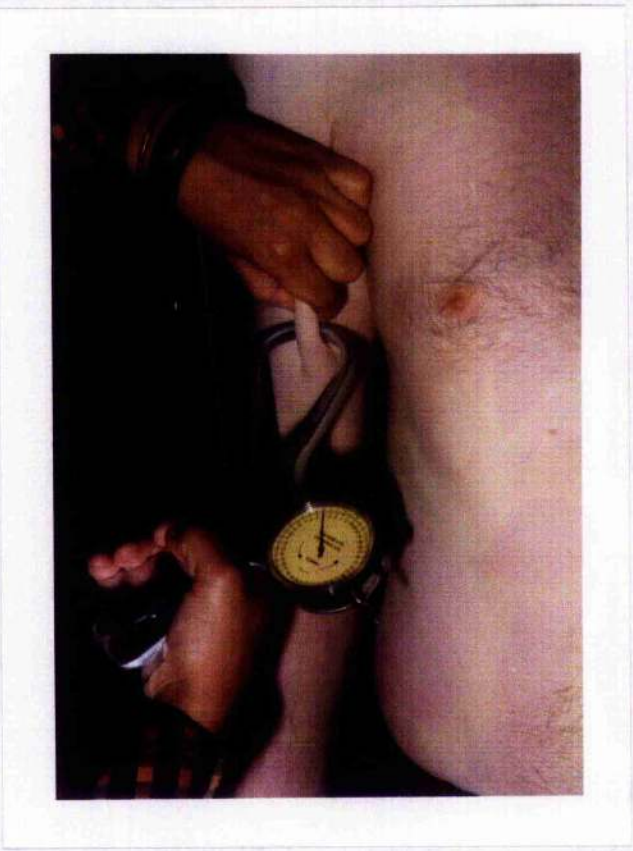
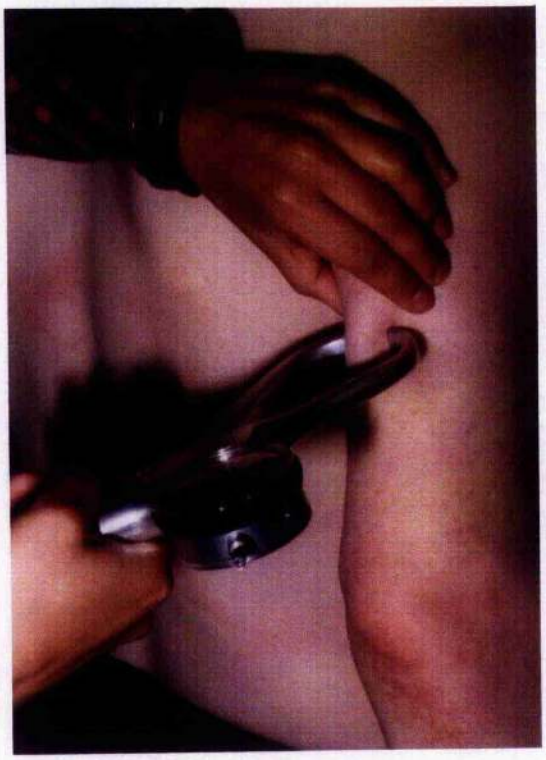


Figure 3.12. Measurement of thickness of folds of adipose tissue and skin.
Biceps and subscapular skinfolds.



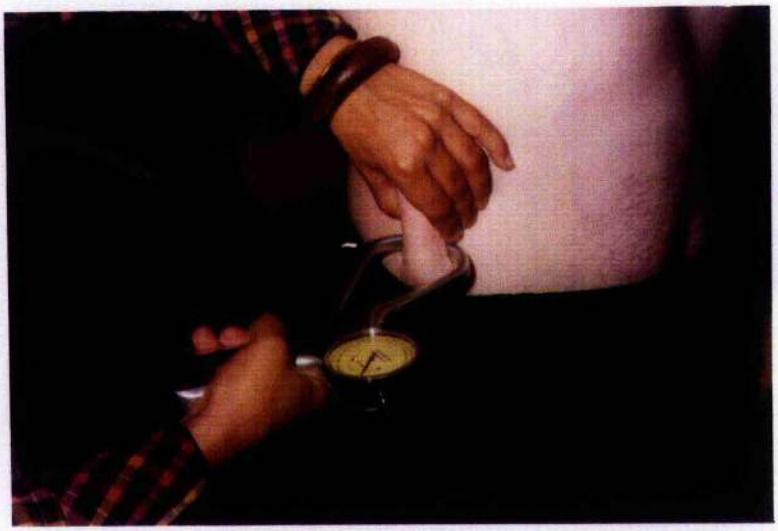


Figure 3.12. (continued). Triceps and suprailiac skinfolds.

3. ANALYSIS OF DATA.

3.1. Comparison between methods.

Estimates of B.C. by each method were compared against each other using the method of comparison described by Bland & Altman, 1986 and by Kramer & Feinstein, 1981 whose approach is based on graphical techniques and calculations.

The Bland & Altman's approach. The first step was to plot the data, of each method against the other, for fat% and FFM and for each sex, and draw the line of equality to have an idea of the degree of agreement between measurements.

The second step was to plot of the difference between each two methods against their mean to illustrate in a more detailed fashion the pattern of how any of the methods was likely to differ from the others, such as any possible relationship between the difference and the mean value, i.e., tendencies towards an increase or decrease of the difference as a function of the mean value. Another advantage with this plot is to find, when it exists a cut off point, i.e., a value (or a range of values) at which something different happens.

The mean value is used as the best estimate of the unknown 'true' value and the difference between two methods, as indicator of the 'measurement error'.

The agreement may be summarized by calculating the bias, estimated by the mean difference (δ) and the standard deviation of the differences (SDd). Most of the differences (95%) are expected to lie between the limits $\delta \pm 2$ SDd if the differences are normally distributed and are referred to as "limits of agreement". Such differences will be likely to follow a normal distribution because a lot of the variation between subjects are removed with this procedure. If there is a consistent bias an adjustment can be made by adding up or subtracting the mean difference to adjust both methods.

The limits of agreement are only estimates of the values which apply to the whole population. A different sample would give different limits. Therefore, the confidence interval (C.I.) of the mean difference was calculated; it refers to the range of values where the mean value will lie with probability of 98%. This value was used because 3 methods are being compared. The C.I. was obtained calculating the standard error of the difference (SEd) between methods which is:

$$SEd = \sqrt{\frac{SDd^2}{n}}$$

A level of significance of 0.02% was then calculated by finding the appropriate point of the t distribution (two tails) with n-1 degrees of freedom and the C.I. was:

$$\delta \pm (t_{0.98} * SEd)$$

When this range of values does not include zero, there is a significant difference between the methods being compared. The width of the interval helps to interpret the sample size.

The maximum difference shall also be included in the description of the results.

The Kramer & Feinstein's approach: the concordance index (R_1 æ). Another approach to measure agreement between methods was the calculation of the concordance index (R_1 æ) also known as the intraclass correlation coefficient proposed by Kramer & Feinstein, 1981.

In the present study it was wanted to know the extent to which the comparison between each two methods yield the same result, or are concordant with each other. The concordance index expresses quantitatively the comparison (degree of agreement) between the outputs of each two methods.

Bland & Altman, 1986 and Kramer & Feinstein, 1981 have discussed on the relationship between two methods as evaluated by the correlation coefficient, and other indexes of trend, and both have concluded that they represent the strength of the tendency for changes in one variable to be reflected by changes in the other; they can be used to describe relatedness or mutual tendency between two variables but not the agreement between two variables, and so their use would be inappropriate for the present study.

The above mentioned authors have put forward the following considerations:

- Bias is totally ignored by correlation statistics; two variables (in this case two methods) will have perfect agreement only if the results of both methods are the same. i.e., the points lie along the line of equality; but there would be perfect correlation if one of the variables changes exactly as function of the other. i.e., the points lie along any straight line. Two variables may be very closely related and yet never agree; furthermore, two variables that have a perfect inverse correlation will obviously not be concordant.

- The scale of measurement does not affect the correlation but it certainly affects the agreement. Two variables may or may not be expressed in similar scales of measurement (nominal/existential, ordinal or dimensional).

- Correlation depends on the range of the true quantity in the sample.

- The test of significance is irrelevant to the question of agreement.

- Data which seem to be in poor agreement can produce quite high correlations and yet it could conceal considerable lack of agreement.

- Concordance between two variables will show the extent to which one of the variables can serve as a substitute for the other.

- Unfortunately, the statistics of trend have become so entrenched that the special qualities and advantages of concordance indexes have often been unrecognized.

In order to avoid confusion between the most common way in which correlation coefficient (r) and concordance index (R_1) are expressed, in this document, the symbol R_1 shall always be accompanied by the symbol **æ**, i.e. **R_1æ**.

Principle. The R_1æ combines a measure of correlation with a test in the difference of means. It assesses not only similarity of slopes, but also similarity of intercepts. Thus, if one variable is systematically higher or lower than the other, R_1æ will be correspondingly reduced. R_1æ can vary between -1 and +1, with higher scores reflecting increasing method or observer agreement.

Mathematical definition. R_1æ derives its mathematical definition from a repeated-measures analyses of variance (ANOVA) model. The general idea is that the total variance among the various measurements or judgements is apportioned among three sources: the differences among methods, the differences among subjects, and a remaining 'unexplained' residual or error variance. The pair-wise agreement between two methods employs the equation:

$$R_1 = \frac{msS - msE}{msS + msE + 2(msM)}$$

where:

msS = mean square (i.e., variance) due to differences among subjects;

msM = mean square due to differences among methods;

msE = mean square due to residual (error) variance, providing the number of subjects is large enough.

R_1æ will be maximized if msS is high relative to msM and msE, i.e., if variance due to differences among subjects is large compared

to variance due to differences between methods and error variance. The quantitative significance of R_iæ depends on its absolute magnitude; a minimum value of 0.75 has been recommended (Kramer & Feinstein, 1981).

NOTE. As it has been said, the true values of the body components are not known for sure by either of the methods employed, all of them have their own assumptions. Neither is known which method is the most reliable and therefore no reference method was employed.

In this document the description of the results when comparing methods is expressed as one method being higher or lower than the other.

To make it simple, the potassium (K) and skinfold (SkF) methods were always subtracted from densitometry (D) and the SkF method from K. But it should be remembered that the judgement should be impartial.

3.2. Use of Regression Standards to Predict FFM from TBK.

The use of a regression equation relating FFM [kg] (by densitometry) and the amount of measured potassium (K) [mmol] was tried in order to find out whether, for this population, the calculations of the FFM by K could be better estimated by the use of a regression standard instead of a ratio standard.

The use of ratio standards has been criticized by Tanner (1949), Katch (1973), Katch & Katch (1974) and, more recently, by Winter & Maughan, 1991 and by Nevill et al, 1992 because "its use is theoretically fallacious and in practice (except under very special circumstances), misleading". These simple ratio standards have been used in physiology to facilitate the comparison of measurements recorded from individuals of different sizes; by dividing by an appropriate body size variable, it is assumed that differences in the physiological variable due to the subject's size will have been removed.

The fallacy consists in that a regression line does not normally pass through the origin. The straight line equation: $\mathbf{Y = bx + a}$ will only coincide with the ratio line $\mathbf{y = kx}$ when the two regression coefficients **b** & **k** are equal, and when **a** is zero (an equation should not be taken to unrealistic regions). This condition is only true when the two variables are perfectly proportional and it can be put in another form: the prediction from the two standards will be the same when the correlation coefficient (r) that relates the two variables (TBK and FFM, in this case) is numerically equal to the coefficient of variation (CV) of the "x" variable divided by the CV of the "y" variable: $\mathbf{r = CV_x / CV_y}$ (Tanner, 1949).

It is a common practice, including this study, to use the ratio standard as a means for calculating FFM on the assumption that the amount of potassium of the FFM is constant for changing size and that it differs between sexes. However, the fact that different conclusions and predictions have been found when the amount of potassium is expressed per kilogram of FFM (K/FFM) may be explained, in part, by complications caused by the use of the K/FFM ratio.

The K/FFM was assumed as a constant value by Forbes et al, 1961 when they found a mean value, of 4 cadavers chemically analysed, of 68.1 mmol/kg. From then on, a ratio is obtained by dividing the amount of potassium by the FFM estimated by any of the indirect methods and most studies report different mean values for various studies and different mean values for females and for males.

It was then decided to try in this study a linear adjustment method to scale how TBK might best be adjusted for differences in FFM and study whether there is a difference between males and females in the K/FFM.

NOTE. Although physiologically the amount of K depends on the FFM, mathematically the variable to be predicted is FFM and TBK the predicting variable as it will be appreciated in the pertinent graphs.

3.3. Comparison of the straight-line regression models between sexes to predict FFM from TBK.

A comparison of the females' and males' straight lines to predict FFM from body potassium was performed. The statistical approach followed was that suggested by Klenbaum & Kupper, 1978 to compare two straight-line regression models.

The procedure consists in four steps:

a. comparison of slopes: test of parallelism, which involves computing the following test statistic:

$$Z = \frac{\hat{\beta}_{\phi M} - \hat{\beta}_{\phi F}}{\sqrt{S^2_{\hat{\beta}_{\phi M}} + S^2_{\hat{\beta}_{\phi F}}}}$$

where:

$$S^2_{\hat{\beta}_{\phi M}} = \frac{S^2_{Y|X_M}}{(n_M - 1) S^2_{X_M}}$$

estimates the variance of the estimated slope B_{1M} for males and

$$S^2_{\hat{\beta}_{1F}} = \frac{S^2_{Y|X_F}}{(n_F - 1)S^2_{X_F}}$$

estimates the variance of the estimated intercept B_{0F} for females.

b. comparison of intercepts, which involves computing the following test statistic:

$$Z = \frac{\hat{\beta}_{0M} - \hat{\beta}_{0F}}{\sqrt{S^2_{\hat{\beta}_{0M}} + S^2_{\hat{\beta}_{0F}}}}$$

where:

$$S^2_{\hat{\beta}_{0M}} = S^2_{Y|X_M}\left[\frac{1}{n_M} + \frac{\bar{X}^2_M}{(n_M - 1)S^2_{X_M}}\right]$$

estimates the variance of the estimated intercept B_{0M} for males and

$$S^2_{\hat{\beta}_{0F}} = S^2_{Y|X_F}\left[\frac{1}{n_F} + \frac{\bar{X}^2_F}{(n_F - 1)S^2_{X_F}}\right]$$

estimates the variance of the estimated intercept B_{0F} for females.

c. Coincidence of the lines in slope and intercept. If the two regression models are the same both reduce to a general equation for both sexes.

When the tests of comparison of slope and intercept are both not rejected, one can conclude that the two lines are coincident. However, the significance level (α) of the two tests combined is greater than for each separate test. To get around this difficulty α is divided by 2 for each separate test to guarantee an overall significance level of no more than α.

d. Comparison of correlation coefficients, to determine whether or not the strength of the straight line relationship was the same for females as for males. This was computed with the following test statistic:

$$Z = \frac{\frac{1}{2}\log_e \frac{1+r_M}{1-r_M} - \frac{1}{2}\log_e \frac{1+r_F}{1-r_F}}{\sqrt{\frac{1}{n_M-3} + \frac{1}{n_F-3}}}$$

this equation includes the Fisher's Z transformation of the population correlation coefficients for each sex, respectively, which is necessary to test the null hypothesis; 1/(n-3) stands for the variance.

The appropriate null hypotheses H_o for comparison of slope, intercept and correlation coefficient were given by 'equality' between females and males.

The significance level to reject H_0 was $\alpha = 0.05$; or else, in using Z to perform two sided tests for equality, $|Z|$ had to exceed $Z_{0,975}$ to be rejected.

3.4. Derivation of equations to predict body density from skinfolds. Stepwise multiple regression analyses were performed to predict body density including sex, age and all the anthropometric variables. The equation giving the lowest standard error of the estimate was chosen.

Linear regression equations were also performed to predict body density from the logarithm of single and all possible combinations of the four measured skinfolds.

NOTE. The results of total body water are not included in this document because the determination of the concentration of the deuterium oxide of the saliva samples has not yet been done.

RESULTS

1. GENERAL CHARACTERISTICS OF THE VOLUNTEERS.

A total of 157 subjects participated in this study, 79 females and 78 males. Their mean general characteristics: age, body mass, height, body mass index and fat proportion estimated by the Durnin and Womersley, 1974 formulae, are presented in table 3.2. (In table 2.1. is presented the general data of all the variables studied).

2. DESCRIPTION OF THE RESULTS OF BODY COMPOSITION.

Tables 3.3.A. & B. present a complete description of the results of fat%, fat mass and fat free mass (FFM) obtained by the three methods: densitometry (D), skinfolds (SkF) and total body potassium (TBK) (calculated using 60 and 68 mmol/kg K/FFM for females and males, respectively); the data includes the values: mean $\pm$ 1 standard deviation (S.D.), the smallest, the largest and percentiles 25, 50 and 75%.

Comparisons of fat% and of FFM between methods are shown in figures 1 to 8, appendix (App.) 2. The Concordance Index ($R_1æ$) and the description of the results of each pair of methods are also shown.

Tables 3.3.A. & B. and figures 1 & 2, App. 2, show that the fat% by SkF is higher than D ($p < 0.001$) in most cases (61/78 females and in 62/78 males which account for about 78% of all subjects). The $R_1æ = 0.05$ was too low in both sexes. There is a bias; SkF gives higher values than D on most cases.

Alternatively for FFM, SkF gives lower values than D on most subjects ($p < 0.001$). It can be seen in figures 3 & 4, App. 2 that most points are below the equality line. $R_1æ$ values were low, i.e., 0.23 & 0.37 for females and males, respectively.

The comparison between fat% by densitometry (D) and by potassium (K) for females (n=26) is shown in table 3.3.A. and in fig. 5, App. 2. It can be seen that the K method gave systematically lower values than D (23/26 cases); the $R_1æ$ was = 0.07. There was woman with zero fat% by K and about 10% fat by D, she was very athletic & lean-muscular, the value by D would be low but possible for a female but the value obtained by K would not, because at least the essential fat should exist.

For males, the comparison between D & K for fat% are shown in table 3.3.B. and in figure 6, App. 2, the $R_1æ$ was = 0.48, the mean values for both methods were almost equal, but there were large differences. It was noticeable a negative fat% value by K which would obviously be impossible, and a subject with a fat% value by D of about 22% fat and 30% by K.

	FEMALES (n = 79)	MALES (n = 78)
AGE	26.6 ± 7.1	26.2 ± 8.1
(decimal yrs.)	(16.0-63.0)	(17.0-53.0)
BODY MASS	55.4 ± 6.4	68.8 ± 8.2
(kg)	(40.2-68.2)	(53.3-92.2)
HEIGHT	165.2 ± 6.1	179.7 ± 7.3
(cm)	(150.2-178.9)	(162.5-195.8)
Body Mass Index	20.2 ± 1.69	21.3 ± 2.38
(kg/m^2)	(16.1-24.2)	(17.0-29.7)
Fat %	22.0 ± 4.03	12.7 ± 3.60
	(10.2-31.2)	(6.7-27.0)

Table 3.2. General characteristics of the volunteers. Values represent mean ± S.D. and range in brackets. Three women and six males were older than 39 years.

METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
A.1 FAT %						
D (n=78)	19.6 ± 4.55	4.8	16.1	20.0	22.9	28.4
SkF (n=79)	22.0 ± 4.03	10.2	19.6	21.9	25.0	31.2
K (n=27)	14.6 ± 6.02	0.4	10.1	13.0	20.3	24.7
A.2 FAT MASS [kg]						
D (n=78)	11.0 ± 3.11	2.6	8.7	10.9	13.2	18.3
SkF (n=79)	12.3 ± 3.03	4.3	10.3	12.4	14.2	18.8
K (n=27)	8.4 ± 3.73	0.2	5.7	7.3	11.9	15.3
A.3 FAT FREE MASS [kg]						
D (n=78)	44.4 ± 5.04	31.7	41.4	44.7	47.6	54.1
SkF (n=79)	43.1 ± 4.62	30.2	40.3	42.4	47.2	52.0
K (n=27)	48.9 ± 5.38	35.2	45.9	49.2	52.3	60.3

Table 3.3.A. Description of the results of body composition for females.

METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
B.1 FAT %						
D (n=78)	10.7 ± 3.84	4.5	7.8	10.1	13.0	21.9
SkF (n=78)	12.7 ± 3.61	6.7	10.1	12.4	14.9	27.0
K (n=38)	9.6 ± 6.42	-2.0	5.3	9.7	12.9	30.8
B.2 FAT MASS [kg]						
D (n=78)	7.4 ± 2.98	2.7	5.6	7.1	8.8	17.2
SkF (n=78)	8.8 ± 3.05	3.9	6.7	8.6	11.0	21.2
K (n=38)	6.7 ± 4.84	-1.2	3.2	6.4	9.3	24.2
B.3 FAT FREE MASS [kg]						
D (n=78)	61.4 ± 7.28	48.1	55.3	61.3	66.6	83.1
SkF (n=78)	59.9 ± 6.83	48.8	54.8	59.1	64.3	80.8
K (n=38)	61.9 ± 8.02	47.7	54.8	62.4	68.0	79.2

Table 3.3.B. Description of the results of body composition for males.

For males, it is shown in table 3.3.B. and in figure 8, App. 2, that the mean FFM values obtained by D and K were very similar. The $R_1æ$ = 0.86, which represents a good concordance and was the best of all comparisons. There is a symmetry of the values, i.e., there are very similar number of values above and below the equality line and with similar distance; the mean and the distribution of values are practically the same; although it can be seen that most of the values do not lie along the line.

For females, the comparison between FFM by D and by K showed a bias: K gave higher values than D in 23 out of 27 cases (table 3.3.A. and figure 7, App.2), the $R_1æ$ was = 0.12.

The distribution of the differences are shown in tables 3.4.A. & B., these data include the values: mean difference ± 1 standard deviation (S.D.), the minimum, maximum and the percentiles 25, 50 and 75%.

Tables 3.5.A. & 3.5.B. show the 98% confidence interval (C.I.) and the limits of agreement for fat% and for FFM, for females and males, respectively. (The C.I. is the range of values where the mean value will lie with probability of 98%).

Figures 9 to 20, App.2, show the plots of difference on mean between each pair of methods for fat% and for FFM, the three lines represent the mean difference ± 2 S.D.. It is necessary for the interpretation of these results to examine the plots.

Comparisons between each pair of methods are: first D and SkF; D and K and lastly K and SkF. Each figure is described making reference to the data of tables 3.4. and 3.5.

METHOD	MEAN ± S D	min	p 25%	p 50%	p 75%	max
A.1 DIFFERENCES OF FAT% BETWEEN METHODS						
D - SkF (n=78)	-2.4 ± 3.32	-11.4	-4.5	-1.9	-0.3	5.9
D - K (n=26)	5.7 ± 4.76	-4.2	3.2	6.1	8.0	15.4
K - SkF (n=27)	-8.0 ± 4.43	-17.8	-10.9	-8.1	-5.2	3.2
A.2 DIFFERENCES OF FFM [kg] BETWEEN METHODS						
D - SkF (n=78)	1.3 ± 1.85	-3.4	0.1	1.0	2.5	6.4
D - K (n=26)	-3.3 ± 2.76	-8.9	-4.3	-3.4	-1.8	2.2
K - SkF (n=27)	4.6 ± 2.56	-2.0	3.3	4.9	6.1	10.8

Table 3.4.A. Differences of fat% and FFM [kg] between methods for females.

METHOD	MEAN ± S D	min	p 25%	p 50%	p 75%	max
B.1. DIFFERENCES OF FAT% BETWEEN METHODS						
D - SkF (n=78)	-2.0 ± 2.93	-10.5	-3.9	-2.2	0.06	3.8
D - K (n=38)	0.8 ± 4.41	-10.2	-2.3	1.3	3.6	9.3
K - SkF (n=38)	-2.8 ± 4.72	-15.1	-4.7	-3.0	0.5	8.1
B. 2 DIFFERENCES OF F F M BETWEEN METHODS						
D - SkF (n=78)	1.4 ± 2.14	-2.8	-0.04	1.5	2.6	8.1
D - K (n=38)	-0.5 ± 3.16	-6.5	-2.3	-0.8	1.4	7.4
K - SkF (n=38)	2.0 ± 3.36	-5.9	-0.3	2.2	3.4	11.7

Table 3.4.B. Differences of fat% and FFM [kg] between methods for males.

METHODS	98 % C.I. FOR THE MEAN DIFF.	LIMITS OF AGREEMENT (MEAN ± 2 SD)
A.1. F A T %		
D - SkF (n=78)	-3.3 to -1.5	-9.0 to 4.2
D - K (n = 26)	3.3 to 8.0	-3.9 to 15.2
K - SkF (n=27)	-10.1 to -5.9	-16.9 to 0.8
A. 2. FAT FREE MASS [kg]		
D - SkF (n=78)	0.8 to 1.8	-2.4 to 5.0
D - K (n = 26)	-4.6 to 1.9	-8.8 to 2.2
K - SkF (n=27)	3.4 to 5.8	-0.5 to 9.7

Table 3.5.A. Confidence interval for the mean difference and limits of agreement for females.

METHODS	98 % C.I. FOR THE MEAN DIFF.	LIMITS OF AGREEMENT (MEAN ± 2 SD)
B.1. F A T %		
D - SkF (n=78)	-2.8 to -1.2	-7.9 to 3.8
D - K (n = 38)	-1.0 to 2.5	-8.1 to 9.6
K - SkF (n=38)	-1.0 to -4.7	-12.3 to 6.6
B.2. FAT FREE MASS [kg]		
D - SkF (n=78)	0.9 to 2.0	-2.8 to 5.7
D - K (n = 38)	-1.7 to 0.8	-6.8 to 6.3
K - SkF (n=38)	0.6 to 3.3	-4.7 to 8.7

Table 3.5.B. Confidence interval for the mean difference and limits of agreement for males.

3. COMPARISON BETWEEN BODY COMPOSITION RESULTS CALCULATED FROM THE METHODS OF DENSITOMETRY AND SKINFOLDS.

The methods of D and SkF gave similar results between sexes. The mean difference of fat% was 2.4 units for females and 2.0 units for males, SkF giving higher values than D on most subjects. The S.D. was slightly higher for females (3.3 units) than for males (2.9 units). The mean difference was not a constant value along the fat% values, but the 98% confidence interval (C.I.) for the mean difference, where the mean difference value is liable to lie, ranges from -3.3 to -1.5 units for females and from -2.8 to -1.2 units for males; it can be seen that these ranges do not include zero, therefore there is an obvious significant difference between these two techniques and some adjustment should have to be done in order to have more similar values. There might be differences as high as 11 units; however, most of the differences will lie between about 4 units below to 8 units above, for both sexes (figures 9 & 10, App. 2).

For these data, there was no obvious relation between the difference and the mean. For a given mean value of fat% there were various differences of fat% between these two methods.

One of the hypothesis of this study was that leaner individuals could have greater differences between these two methods, than the not so lean or "normal" subjects. (This, because the subjects from whom the equations used to estimate B.C. of this study were derived, were preponderantly moderately sedentary, although there were some volunteers from health clubs, sports organizations and a ballet company who could have had low fat%, Durnin & Womersley's, 1974). This was not found, leaner subjects of both sexes, had fat% differences in about the same range of values as the rest of the group.

The narrow width of the C.I. encountered for these groups of subjects, about 1.5 units, was acceptable to conclude that the sample size, was big enough. The wide intervals of agreement and the low concordance values ($R_1æ$ = 0.05), for both sexes, show that the agreement between this two methods in their fat% prediction is not so good.

For the FFM data, females had a mean difference between D & SkF of 1.3 kg and males 1.4 kg. The S.D. was slightly higher for males (2.1 kg) than for females (1.9 kg). These mean differences were not a constant bias throughout the FFM mean values; the C.I. for the mean difference ranged from about 1-2 kg of FFM. The limits of agreement showed that the SkF technique may be about 3 kg above to 6 kg below D, but some individuals may have discrepancies of FFM between methods of up to 8 kg. The concordance values were $R_1æ$ = 0.23 & 0.37 for

females and males, respectively (figures 11 & 12, App. 2). These results reinforce the statement that these two methods significantly differ from each other, for both sexes.

4. COMPARISON BETWEEN BODY COMPOSITION FIGURES CALCULATED FROM THE METHODS OF DENSITOMETRY AND POTASSIUM.

The comparisons between D and K were different between sexes.

For females, the estimation of B.C. assuming a K content of the FFM (K/FFM) of 60 mmol/kg, gave big discrepancies with respect to D. The mean difference of fat% was 5.7 $\pm$ 4.76 units, K giving lower values than D. Figure 13, App. 2, shows the plot of the difference of fat% between these methods against their mean. Twenty three out of 26 women had lower values by K, ranging from 0.7 to 15.4 units. The other 3 women with D values above K had values from 1.1 to 4.2 units of fat%. The C.I. for the mean difference was between 3.3 to 8 units, this wide range may show that the number of subjects was not enough; this range does not include zero showing the bias of the K method to give lower fat% values than D, the bias was not constant along fat% values. The limits of agreement, where most of the differences will lie, ranged from -3.9 to 15.2 units of fat%.

Some relation between difference and mean value was found. For those differences above zero (positive differences: D higher than K), there seems to be an increase in the difference between methods as the mean fat% value diminishes. Note that at a mean fat% of ≈18, there may be a change. Most females with fat% values below 18, have an overestimation of fat% by D above the mean error measurement (about 6), and most females with fat% above 18, have an overestimation of fat% by D below average. If the mean difference could be brought to zero, i.e., by using a higher value of K/FFM leaner females would have higher values by D and the others higher values by K.

The range of differences of fat% found in this group of women was from -4 to +15. It is interesting to note that this range was also found at the mean fat% value of about 18. Then, although there seems to be a trend, there are exceptions.

For the FFM data of females (figure 14, App. 2) it was noticed that there is a bias, the K method gives lower values of FFM than D by a mean value of 3.3 kg but it can be as high as 9 kg; therefore the bias was not constant. The C.I. for the mean difference ranged from -4.6 to 1.9, making the bias apparent; its large width may indicate a too small sample size. The limits where most of the differences lied ranged from -8.8 to 2.2.

There seems to be some relation between difference and mean; the trend is to greater differences as FFM values increases, but it can be seen that at a mean value of about 45 kg the range of differences is almost wholly included. Then, the trend cannot be considered as a fact.

It can be summarized for the comparison of fat% and FFM between the methods of D and K in females, that the ranges of the C.I. and of the limits of agreement are wide, probably reflecting the small sample size (n= 26) and the great variation of the differences. The discrepancies between these two methods are considerable and the degree of agreement (concordance=0.1) is too low to be accepted.

The relationship between differences and means, may imply that the actual value of fat% and of FFM should be taken into account for the calculation of B.C. by the K method. Most women with less fat% and some with more FFM, had greater differences. This calls the attention to the use of a unique & constant value for all females (in this case 60 mmol per kg of FFM was used for all).

The fact that a range of differences can be found for a given measurement of fat% and of FFM, may indicate that other physiological factors besides the values of fat% or of FFM **per se**, have an influence and may help to explain the differences.

The individuals with largest differences were separately studied to look at any special characteristic that could give a reason to explain the fact. Nothing was found.

Males, in comparison with females had lower mean differences between D & K; in fact, it can be seen that it was almost zero but the distribution of differences are too wide to accept that this two methods give similar B.C. results. For example, it can be seen that there are differences of up to 10 units of fat% and up to 7.4 kg of FFM.

Figure 15, App.2 shows the difference between the methods of D and K against their mean for fat% for males; the mean fat% difference between methods was 0.8 units with a C.I. between -1.0 to 2.5, D giving higher values. This is not a significant difference, but the distribution of the differences show discrepancies as high as 10 units; the limits of agreement where most of the differences will lie, ranged from -8.1 to 9.6 units.

There is a relation between difference and mean. Those subjects with less than about 10% fat, have their fat% estimates higher by D and from this value up there is a switch as most differences change to lower estimates by D; in other words, there is a trend and the use

of a constant single value to estimate B.C. from K on all subjects must be questioned.

However, it can also be seen that for a given mean value a wide range of differences may be found; i.e., around the mean fat% value of 11.5, there are 8 subjects with differences between methods from -5 to 4 units, this range is not too far from ± 1SD (4.4), so that about 66% of the differences lie between these limits.

A very gross example using these figures would be a man with a true fat% value of 11, who could have his estimation of fat%, following the assumptions used, as low as 6% or as high as 15%.

Figure 16, App.2, shows the FFM difference between D and K against its mean for males. A mean difference value of 0.5 kg was found, with a C.I. for the mean difference between -1.7 to 0.8 kg, the K method giving higher values. The mean difference is not significant; however, the limits of agreement, where 95% of the differences will lie, ranged from -6.8 to +6.3 kg and the range of differences showed values as high as 7.4kg. Although the index of concordance was high (0.86), the values herein presented show that, for some individuals, the agreement between these two methods is not so good.

It can be appreciated in figure 16, App.2, that the scatter of differences increases from about the mean FFM value of 60 kg. From this point, K gives higher FFM values than D in most cases and to a greater extent than for values below 60 kg. Probably then, the limits of agreement, for those values below 60kg could be narrower.

Summarizing, from the comparison between these two methods it can be said that being the C.I. so narrow, the number of subjects studied was enough. The mean differences for fat% and for FFM estimated by D and by K were non significant, however the limits of agreement and the ranges of the differences for both measures were wide and comparable to those of females. For fat% the concordance was low ($R_1æ$ = 0.48) and so it could be concluded that there is lack of agreement between these two methods; however, the concordance for FFM is $R_1æ$ = 0.86 which is a fairly high value. The reason for the difference in the results of the concordance index between fat% and FFM lies in that FFM values are greater than those of fat%. Still, it looks as the methods do not agree that well, as it could be seen that, for fat%, besides the wide width of the limits of agreement, there is some relationship between difference and mean values; this could mean, the same as for women, that either the assumed density of the FFM (1.100 g/cm^3) or the amount of potassium of the FFM (68 mmol/kg FFM) or both, may differ in subjects with different body

builds. Both, the density of the FFM and the amount of potassium of the FFM are not known but, because there is a trend, the use of a constant value to estimate FFM and fat% from the potassium measurement should be more deeply analysed.

5. COMPARISON OF THE BODY COMPOSITION FIGURES CALCULATED FROM THE METHODS OF POTASSIUM (K) AND SKINFOLDS (SkF).

Figure 17, App.2, shows the difference of fat% between K and SkF against their mean for females. There is a bias of the SkF method to give higher fat% values than K by a mean value of 8.0 units; the C.I. for the mean difference is -10.1 to -5.9 units, this wide range makes apparent the small sample size. The highest difference was -18 units of fat% and the limits of agreement show that SkF may be from 1 unit below to 17 units of fat% above K for 95% of the sample, which would be unacceptable. The graph shows a more obvious trend between difference and mean fat% for SkF and K than for D and K; the trend shows that the difference between methods becomes lower as mean fat% values increase. It can also be appreciated that at a mean value of ≈ 20% fat, there is a change; those females with a mean fat% values below 20 units, present a difference between methods which is lower than the mean difference and those females with fat% above 20, present a difference larger than the mean difference.

Figure 18, App.2, shows the comparison between K and SkF for FFM for females, the results showed that SkF gives lower FFM values by a mean value of 4.6kg, the bias is not constant. The C.I. for the mean difference is between 3.4 to 5.8; the maximum difference was 11kg and the limits of agreement for the 95% of the sample, ranged from -0.5 to 9.7. There was no relationship between the difference and the mean.

The results for males comparing fat% by K and by SkF is shown in figure 19, App.2. It was found that SkF gives higher fat% values averaging 2.8 units; the C.I. for the mean difference was -1.0 to -4.7 (K-SkF), then a bias of SkF to give higher fat% values is obvious and the wide width of the C.I. shows that the number of subjects may have not been enough. There were found differences of up to 15 units of fat% and the limits of agreement for the 95% of the sample, were found between -12.3 to 6.6 units. There is also a relationship between difference and mean, SkF giving higher values than K on most males with low fat% values (≈ <13) and lower values on most not so lean subjects. The highest differences are greater values by SkF and are on subjects with a fat% below 10. K gives

higher fat% values than SkF in 10 out of 38 cases, 7 of them with fat% above 14.

Figure 20, App.2, shows the difference of FFM against its mean. The mean difference was 2 kg and its C.I. 0.6 to 3.3. From these results it is clear that there is a bias of SkF to give lower FFM values than K. There were differences as high to almost 12kg. The limits of agreement showed that for 95 % of the sample, SkF may be 4.7 kg above or 8.7 kg below K.

The scatter of the differences increases for mean FFM values above 60kg. It can be seen that below 58kg of FFM differences are between +2.5 and -4kg but around the value of 60kg there are discrepancies between methods from 8 to -6kg and over 60kg from 11 to -4kg. No trend was found.

Summarizing the comparison between K and SkF, it can be said that there is a lack of agreement between these 2 methods, the magnitude of the differences between these two methods were the highest of the 3 comparisons. As SkF are related to D to predict B.C. it was not surprising to find the same type of relationship as between D and K. The relationships between differences and means for fat% show that it is important to review the assumptions on which the estimations of these 3 methods are based.

DISCUSSION.

1. COMPARISON BETWEEN THE DENSITOMETRY AND THE POTASSIUM METHODS.

1.1. Analysis of the disagreement between methods.

The group of subjects that underwent the TBK study were those that voluntarily accepted to do it. Their general characteristics are presented in table 3.6., so that they can be compared with the whole group (table 3.2.).

The mean values of the group of females that did the K study, were just a little bit higher than those of the whole group, studied by D and SkF; however, the differences were non significant. The group of males had almost the same mean values for all the variables presented herein. Therefore, it could be said that for both sexes the groups belong to the same sample as the whole group.

The lack of agreement between D and K, presented in the former section, and the values cited in the literature, from 48 to 70 mmol as the ratio of K [mmol] per kilogram of FFM (TBK/FFM), made necessary to review the physiological assumptions on which each of these two methods are based to estimate B.C.: the constancy of the TBK/FFM [mmol/kg] and the constancy of the density of the FFM.

One of the approaches was to obtain in each subject the TBK/FFM using the amount of K in mmol and FFM got by D. Table 3.7. shows the results, and figure 21, App.2, shows the histograms for females, males and for both sexes.

FFM by D for each subject was calculated assuming that the density of the FFM is 1.100 g/cm^3, therefore these ratios of K to FFM keep the same assumption.

As it can be seen in table 3.7, females presented a higher mean ratio (64.3 mmol/kg FFM) than the one used previously in this study to calculate FFM by K (60 mmol/kg of FFM). The lowest value was 57 mmol per kg of FFM and less than 25% of the females studied (n≈6) had values below 63 mmol/kg. The fact that the mean and median values are around 64 mmol per kg of FFM estimated by D and not about 60, is the reason for such a large difference found between the D and K methods. The mean value of 64.3 mmol/kg obtained in this study coincides with the observation made by Forbes, et al (1968, 1976) that the amount of K/FFM might be lower in females because of a lower ratio exchangeable potassium: total body water (K_e/TBW).

For males, the mean ratio of 68.5 mmol/kg was similar to the value most commonly used and to the mean of 4 adult human cadavers cited by Forbes et al, 1961 & 1976 (68.1 mmol/kg).

It is important to highlight the different mean values for each sex and the wide range of values found: 57 to 75 mmol of K per kg of FFM.

	FEMALES (n = 27)	MALES (n = 38)
AGE	26 ± 4.6	26 ± 8.7
[decimal yrs.]	(19.0-38.0)	(17.0-53.0)
BODY MASS	57.4 ± 5.4	68.5 ± 8.2
[kg]	(40.2-65.6)	(55.9-85.5)
HEIGHT	166.8 ± 5.1	179.7 ± 7.9
[cm]	(154.3-174.4)	(162.5-195.8)
Body Mass Index	20.6 ± 1.63	21.2 ± 2.10
[kg/m^2]	(16.1-24.2)	(17.0-26.5)
Fat [%]	22.7 ± 2.75	12.4 ± 3.64
	(16.5-27.2)	(6.7-27.0)
POTASSIUM [mmol]	2933.0 ± 328.8	4206.4 ± 545.5
	(2112.8 - 3615.4)	(3243.6 - 5387.2)

Table 3.6. General characteristics of the volunteers that performed the potassium study.

SEX	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
FEMALES (n=26)	64 ± 3.7	57	63	65	66	72
MALES (n=38)	69 ± 3.4	60	66	69	71	75
BOTH (n=64)	67 ± 4.0	57	64	66	70	75

Table 3.7. Amount of potassium of the FFM.

It was decided to use the rounded values of 64 for females and for males 69 mmol of K per kg of FFM to re-calculate the fat% and the FFM from the K measurements. These results and the estimations by D are presented in table 3.8. and the difference of fat% and of FFM between K and D in tables 3.9. & 3.10. Tables 3.11. and 3.12. show the 95% confidence interval (C.I.) and the limits of agreement for both sexes for fat% and FFM, respectively.

The mean values were in close agreement; however, it can be seen in table 3.8.A. that for females along the distribution of values of fat%, K gave lower estimates than D up to the percentile 50, thereafter D estimates were higher. For FFM, the distribution of the values showed that K gave slightly higher estimates than D only at the extremes.

For males, table 3.8.B. shows that there is still a negative value of fat%, which is of course impossible. The distribution showed that up to the percentile 25, K estimates for fat% were lower than D estimates and thereafter the opposite happened and the differences seemed to increase as the fat% values increased. FFM values were all in good agreement.

The mean differences of fat% and FFM for both sexes were almost nil; however the 95% C.I. showed a somehow wide range for the mean differences. Comparing the data for females using 64 instead of 60 mmol per kg of FFM, the limits of agreement kept about the same width but using the value of 64 the differences were obviously better distributed above and below zero.

As it can be seen from tables 3.9. & 3.11. and figures 22 & 23, App. 2, the differences of fat% between methods were still large for some subjects; the distribution of differences showed that these can be as high as 8 units and even greater, for both sexes. For males, the use of 68 or of 69 mmol/FFM did not make any important change in what has already been said and for females, the main change is that now the mean difference is almost zero. Now more similar plots can be seen between females and males.

Some relation between the difference and the mean can be seen. Most of the lean subjects (less than 20% fat for females and less than 10% fat for males) have D values higher than K, and most of the normal or of the not-so-lean subjects, have greater values by K than by D. However, there were some very lean individuals, with K values greater than D. It can also be seen that for a given mean value of fat% a wide range of discrepancies can be found. Therefore it is difficult to arrive to any conclusion.

METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
A.1 FAT %						
Densitometry	20.4 ± 4.17	10.5	18.7	20.5	23.7	25.5
Potassium	20.2 ± 5.58	6.6	15.9	18.7	25.3	29.4
A.2 FFM [kg]						
Densitometry	45.6 ± 4.35	32.0	43.2	46.1	48.8	54.1
Potassium	45.5 ± 4.86	33.0	43.1	46.0	48.9	56.5

Table 3.8.A. Body composition estimations using 64 mmol of K per kg of FFM for females (n = 26).

METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
B.1 FAT %						
Densitometry	10.4 ± 3.90	4.5	7.9	9.8	12.6	21.9
Potassium	10.9 ± 6.33	-0.5	6.6	11.0	14.1	31.8
B.2 Fat Free Mass [kg]						
Densitometry	61.4 ± 7.51	48.1	54.3	61.3	66.6	78.7
Potassium	61.0 ± 7.91	47.0	54.0	61.5	67.0	78.1

Table 3.8.B. Body composition estimations using 69 mmol of K per kg of FFM for males (n = 38).

GROUP	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
A. Females (n=26)	0.3 ± 4.49	-8.9	-1.7	0.6	2.5	9.8
B. Males (n=38)	-0.6 ± 4.34	-11.6	-3.6	0.3	2.3	8.0

Table 3.9. Differences of fat% for both sexes (densitometry - potassium) using 64 and 69 mmol K/kg FFM

GROUP	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
A. Females (n=26)	0.4 ± 3.13	-5.5	-1.4	0	2.2	8.2
B. Males (n=38)	-0.2 ± 2.56	-5.7	-1.3	-0.3	1.0	4.9

Table 3.10. Differences of fat free mass [kg] for both sexes (densitometry - potassium) using 64 and 69 mmol K/kg FFM

METHODS	95 % C.I. FOR THE MEAN DIFF.	LIMITS OF AGREEMENT (MEAN ± 2 SD)
A. FEMALES (n=26)		
D - K	-1.5 to 2.1	-8.6 to 9.3
B. MALES (n = 38)		
D - K	-2.0 to 0.9	-9.2 to 8.1

Table 3.11. Confidence interval (C.I.) for the mean difference and limits of agreement for fat% for both sexes.

METHODS	95 % C.I. FOR THE MEAN DIFF.	LIMITS OF AGREEMENT (MEAN ± 2 SD)
A. FEMALES (n=26)		
D - K	-1.2 to 0.8	-5.3 to 4.9
B. MALES (n=38)		
D - K	-0.6 to 1.4	-5.8 to 6.7

Table 3.12. Confidence interval (C.I.) for the mean difference and limits of agreement for fat free mass [kg] for both sexes.

For FFM, tables 3.10. & 3.12. and figures 24 & 25, App.2, show that the differences can be as high as 5 kg for females and up to 7kg for males which represent about 12 and 11%, respectively, of the mean FFM values. No obvious relation between difference and mean was found. For males, above the mean FFM value of about 58 kg the scatter of differences increased.

In conclusion, it can be said that, if the density of the FFM is assumed to be 1.100 g/cm^3, for females the mean value of 64 mmol of K per kg of FFM does better than the value of 60 mmol/kg for the group, but individual differences are still large. As for males the mean value is just in the middle of the two values used (68 and 69), both values are about the same and there are still some subjects with large differences between methods. The fact that the difference and mean fat%, for both sexes, have some relation, makes one to think that the ratio K/FFM is not a constant for all subjects, as most leaner individuals had fat% values higher by D than by K, and the not-so-lean subjects the other way round.

1.2. Effect of physical activity intensity on the comparison of the results between methods.

Another way in which the comparison between the D & K methods were compared was by grouping the subjects by their physical activity intensity, as it has been suggested by Womersley et al, 1976 that the amount of physical activity, directly related to muscularity, may have an influence on the density and potassium content of the FFM.

Subjects were divided into 3 groups by the length of time devoted to exercise, the level of competence or performance and the intensity:

light (2 hours or less per week of light activities such as: easy walk, very slow jogging, badminton),

moderate (3 to 7 hours per week of activities such as: formal walking, martial arts, badminton, weight lifting, jogging & running, fitness courses, squash, swimming...) and

heavy (more than 8 to 30 hours per week of activities such as those included for moderate intensity plus wrestling, ballet dancing, hockey and mountaineering, to this level would belong all the subjects that were enrolled in professional teams and others that although not professionals, used to train very hard).

It should be pointed out that the activity itself was not the reason to allocate a subject in a certain group. The activities are

mentioned only to show what sports were practiced by the subjects selected to each group.

The general characteristics and the generated calculations of B.C. results of fat% , FFM and the amount of K per kg of FFM (K/FFM, mmol/kg) are presented in tables 3.13.A. & B.

There seems to be some differences in the data of B.C. when subjects were grouped by the intensity of physical activity they perform.

One-way analyses of variance were performed on all variables to study whether the differences were significant. The comparison between groups showed non statistically significant differences on any variable on men and only in the amount of potassium [mmol] and FFM by both methods on females ($p< 0.05$). However, some different mean values between groups could be seen on almost all variables, i.e., age, body mass, body density and the amount of potassium were highest for the heavy activity group of both sexes, the heavy activity female group was slightly taller. As a result, FFM was greatest for the heavy activity groups of both sexes and as fat% was highest for the light activity group, lower for the moderate and even lower for the heavy activity group it could be concluded that the heavy activity groups had more muscle mass than the others, the difference being unimportant to the moderate activity groups but important in comparison with the light activity groups.

	LIGHT (n=7)	MODERATE (n=7)	HEAVY (n=12)
AGE	24.4 ± 2.6	26.3 ± 4.6	27.5 ± 5.1
[yrs.]	(20 - 28)	(21-33)	(20 - 38)
BODY MASS	55.0 ± 8.3	57.8 ± 5.2	58.6 ± 3.1
[kg]	(40.2 - 64.2)	(51.2-65.6)	(53.6 - 62.6)
HEIGHT	165.9 ± 5.1	165.4 ± 4.1	167.7 ± 5.9
[cm]	(157.8-173.8)	(157.0-169.5)	(154.3-174.4)
B M I	20 ± 2.11	21 ± 1.73	21 ± 1.15
[kg/m^2]	(17 - 22)	(18-26)	(19-23)
DENSITY	1.048 ± .0056	1.050 ± 0.0101	1.0561 ± 0.0099
[g/cm^3]	(1.041-1.056)	(1.041-1.070)	(1.045-1.075)
POTASSIUM	2657 ± 365.7	2987 ± 261.3	3062 ± 258.8
[mmol]	(2113 - 3136)	(2746 - 3459)	(2659 - 3615)
FFM [kg]	42.6 ± 5.58	45.2 ± 3.24	47.6 ± 3.16
Density	(32.0 - 48.9)	(41.4 - 49.4)	(41.9 - 54.1)
FFM [kg]	41.5 ± 5.71	46.7 ± 4.08	47.8 ± 4.04
Potassium	(33 - 49)	(42.9 - 54)	(41.5 - 56.5)
Fat [%]	22.4 ± 2.60	21.5 ± 4.45	18.8 ± 4.32
Density	(18.8 - 25.7)	(12.6 - 25.5)	(10.6 - 23.9)
Fat [%]	24.3 ± 4.37	19.1 ± 4.99	18.3 ± 5.81
Potassium	(17.9 - 29.2)	(13.1 - 27.6)	(6.6 - 29.4)
TBK/FFM	62.4 ± 2.97	66.1 ± 4.00	64.3 ± 3.53
[mmol/kg]	(56.8 - 66)	(61.6 - 72.4)	(57.2 - 69.7)

Table 3.13.A. General characteristics of the volunteers by intensity of physical activity for females (n = 26).

	LIGHT (n=5)	MODERATE (n=13)	HEAVY (n=20)
AGE	20.6 ± 2.1	25.4 ± 5.1	28.9 ± 10.6
[yrs.]	(18 - 23)	(17-32)	(18 - 53)
BODY MASS	65.9 ± 11.2	68.5 ± 7.8	69.2 ± 8.0
[kg]	(56.4 - 83.6)	(56.4-80.8)	(55.9 - 85.5)
HEIGHT	182.5 ± 10.6	179.1 ± 5.8	179.7 ± 8.5
[cm]	(169 - 195.8)	(169.5-189.4)	(162.5-195.5)
B M I	20 ± 1.75	21 ± 2.15	21 ± 2.08
[kg/m^2]	(17 - 22)	(18-26)	(18-27)
DENSITY	1.068 ± .0066	1.075 ± 0.0062	1.0774 ± 0.0103
[g/cm^3]	(1.058-1.076)	(1.065-1.085)	(1.049-1.089)
POTASSIUM	3826 ± 573.9	4241 ± 584.5	4279 ± 500
[mmol]	(3243 - 4682)	(3431 - 5197)	(3541 - 5387)
FFM [kg]	56.8 ± 8.25	61.3 ± 8.04	62.5 ± 6.92
Density	(48.2 - 68.8)	(48.1 - 73.5)	(52.2 - 78.5)
FFM [kg]	55.4 ± 8.32	61.4 ± 8.47	62 ± 7.25
Potassium	(47 - 67.8)	(49.7 - 75.3)	(51.3 - 78.1)
Fat [%]	13.5 ± 2.84	10.6 ± 2.71	9.4 ± 4.51
Density	(10 - 17.7)	(6.2 - 15)	(4.5 - 21.9)
Fat [%]	15.5 ± 3.53	10.3 ± 5.31	10.1 ± 7.14
Potassium	(9.5 - 18.8)	(2.8 - 21.2)	(-0.4 - 31.8)
TBK/FFM	67.3 ± 1.42	69.2 ± 3.66	68.4 ± 3.54
[mmol/kg]	(65.7 - 69.3)	(60.3 - 72.8)	(60.2 - 74.9)

Table 3.13.B. General characteristics of the volunteers by intensity of physical activity for males (n = 38).

Tables 3.14.A. & B., below show the mean differences, the 95% C.I. for the mean difference and the limits of agreement for fat% and FFM estimates by D and K for females and males, respectively.

When females and males are grouped by their physical activity, the mean differences, the confidence intervals (C.I.) and the limits of agreement logically change. The number of subjects in the light activity groups of both sexes and the moderate group of females were small and any conclusion derived from such small groups should be tentative until more subjects in each group can be studied.

The mean difference of fat% for the group of females was 0.3 $\pm$ 4.49 and of FFM 0.4 $\pm$ 3.1; however, for the light activity group, K gave higher fat% values than D and, concomitantly gave lower FFM values. For the moderate activity group D gave higher fat% values and lower FFM values and for the heavy activity group, the mean difference was almost nil, but the limits of agreement were the largest of the three groups. All differences however, were not statistically significant.

Males had a mean difference in fat% of -0.6 $\pm$ 4.3 and in FFM of -0.2 $\pm$ 2.6. The light activity group presented a mean difference for fat% of -2.1 $\pm$ 1.9 and 1.3 $\pm$ 1.1 for FFM, so there was a bias of the K method to estimate higher fat% values and, concomitantly lower FFM values or vice versa. For the moderate and heavy activity groups the mean differences were near zero but the limits of agreement were wider than for the light activity group.

It may then be that each group has a different value for the K/FFM (assuming that the density of the FFM is 1.100 g/cm^3), or, alternatively, different values of density of the FFM are needed in order to make the estimates from these both methods more agreeable.

	DIFFERENCE MEAN ± SD (min-max)	95 % C.I. FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT (MEAN ± 2 SD)
A.1. Fat [%]			
LIGHT (n=7)	-1.9 ± 3.68 (-8.9 to 2.5)	-5.3 to 1.5	-9.3 to 5.4
MODERATE (n=7)	2.4 ± 4.82 (-3.3 to 9.8)	-2.0 to 6.9	-7.2 to 12.1
HEAVY (n=12)	-0.4 ± 4.42 (-8.9 to 6.9)	-2.4 to 3.2	-8.4 to 9.3
A.2. FAT FREE MASS [kg]			
LIGHT (n=7)	1.1 ± 1.82 (-1.0 to 4.4)	-0.6 to 2.8	-2.6 to 4.7
MODERATE (n=7)	-1.4 ± 2.86 (-5.7 to 1.8)	-4.1 to 1.2	-1.9 to 1.4
HEAVY (n=12)	-0.2 ± 2.57 (-3.7 to 4.9)	-1.9 to 1.4	-5.4 to 4.9

Table 3.14.A. Differences between density (D) and potassium (K) (D-K) estimates for FFM and fat%, confidence interval (C.I.) For the mean difference and limits of agreement for females (n = 26).

	DIFFERENCE MEAN ± SD (min-max)	95 % C.I. FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT (MEAN ± 2 SD)
B.1. Fat [%]			
LIGHT (n=5)	-2.1 ± 1.87 (-4.2 to 0.5)	-4.4 to 0.3	-5.8 to 1.7
MODERATE (n=13)	0.2 ± 4.79 (-11.6 to 5.1)	-2.7 to 3.1	-9.4 to 9.7
HEAVY (n=20)	-0.6 ± 4.53 (-9.9 to 7.9)	-2.7 to 1.5	-9.7 to 8.4
B.2. FAT FREE MASS [kg]			
LIGHT (n=5)	1.34 ± 1.12 (-0.3 - 2.47)	-0.5 to 2.7	-0.9 to 3.6
MODERATE (n=13)	-0.12 ± 3.43 (-3.7 - 8.2)	-2.2 to 1.9	-7.0 to 6.7
HEAVY (n=20)	0.55 ± 3.30 (-5.5 - 7.8)	-1.0 to 2.1	-6.0 to 7.1

Table 3.14.B. Differences between density (D) and potassium (K) (D-K) estimates for FFM and fat%, confidence interval (C.I.) For the mean difference and limits of agreement for males (n = 38).

Even though the values of the TBK/FFM [mmol/kg] between groups of physical activity were statistically not significantly different, because of the large variation of each group and the fact that, for both sexes, the moderate activity groups presented the highest mean value, probably because the different levels of activity might have variable influences on the B.C. of the subjects undertaking a given activity, the possibility of using different values for the D and for the K content of the FFM for the different groups was explored.

Body Density of FFM was obtained using the equation:

$$d_2 = \frac{m_2}{\frac{M}{D} - \frac{m_1}{d_1}}$$

where:

D = density of the total body;

M = body mass;

m_1 = fat mass;

m_2 = fat free mass*;

d_1 = density of the fat mass (0.9 g/cm^3);

d_2 = density of the fat free mass*;

* based on the new values for the K content of the FFM.

For females, the differences of the K content and of the D of the FFM between groups of physical activity were greater than for males; however, the differences between groups were all non significant for both sexes, because the range of values were too wide and they overlap between groups.

GROUP	K CONTENT OF THE FFM [mmol/kg] (FFM DENS. = 1.100 g/cm³)	DENSITY OF THE FFM [g/cm³] (K CONTENT = 60 mmol/kg FFM)	DENSITY OF THE FFM [g/cm³] (K CONTENT = 64 mmol/kg FFM)
LIGHT (n=7)	62.3 ± 3.01 (57 - 66)	1.091 ± .0118 (1.077 - 1.114)	1.107 ± .0129 (1.092 - 1.132)
MODERATE (n = 7)	66.1 ± 3.97 (61 to 72)	1.078 ± .0125 (1.059 - 1.093)	1.093 ± .0137 (1.072 - 1.109)
HEAVY (n=12)	64.3 ± 3.49 (57 - 69)	1.084 ± .0120 (1.067 - 1.111)	1.099 ± .0138 (1.080 - 1.129)
ALL (n = 26)	64.2 ± 3.65 (57 - 72)	1.084 ± .0128 (1.059 - 1.114)	1.099 ± .0141 (1.072 - 1.132)
	p = 0.16	p = 0.2	p = 0.2

Table 3.15.A. Potassium content of FFM based on assumed density of 1.1 g/cm³ and density of FFM based on assumed values for potassium content for females (n=26).

GROUP	K CONTENT OF THE FFM [mmol/kg] (DENSITY OF THE FFM = 1.100 g/cm³)	DENSITY OF THE FFM [g/cm³] (K CONTENT = 68 mmol /kg FFM)	DENSITY OF THE FFM [g/cm³] (K CONTENT = 69 mmol/kg FFM)
LIGHT (n=5)	67.3 ± 1.47 (65 - 69)	1.102 ± .0053 (1.095 - 1.108)	1.106 ± .0055 (1.099 - 1.113)
MODERATE (n=13)	69.1 ± 3.66 (60 - 72)	1.096 ± .0138 (1.084 - 1.132)	1.100 ± .0141 (1.087 - 1.137)
HEAVY (n=20)	68.4 ± 3.54 (60 - 74)	1.099 ± .0130 (1.077 - 1.132)	1.103 ± .0133 (1.081 - 1.137)
ALL (n = 38)	68.5 ± 3.37 (60 - 74)	1.098 ± .0124 (1.077 - 1.132)	1.099 ± .0141 (1.072 - 1.132)
	p = 0.6	p = 0.7	p = 0.7

Table 3.15.B. Potassium content of FFM based on assumed density of 1.1 g/cm³ and density of FFM based on assumed values for potassium content for males (n=38).

It can be seen in table 3.15.A. that the values of density of the FFM based on 60 mmol per kg of FFM for females are unlikely to be true for this group of women; they are too far away from the assumed value of FFM density of 1.100 g/cm^3; instead, the values using 64 mmol, seem more reasonable. For males, 68 or 69 mmol/FFM are both in good agreement with the established value of 1.100 g/cm^3.

The light activity groups have a lower ratio K/FFM and it might be that the obtained values could be used but there are subjects in these groups with ratio values as high as those for the heavy activity groups and besides, the values were not significantly different between groups.

The mean values of the amount of K and density of the FFM could be used for different groups of intensity of physical activity if at least there were a trend of the TBK/FFM to increase and a trend of the density of the FFM to decrease. But as it can be seen in the above tables, the moderate activity group had the highest K/FFM and the lowest density of the FFM in both sexes; therefore this classification does not help.

Because the above classification of physical activity does not make any difference of K/FFM between subjects, another classification of the physical activity as indicator of muscularity was tried. This was done by grouping subjects by the type of physical activity they performed: strength, endurance or mixed. Under **strength**: weight lifters, sprinters and middle distance runners (400, 800 & 1500), rugby players, wrestlers, height jumpers, rowers, cyclers & ballet dancers were included. Under **endurance**: long distance runners & mountain climbers. Under **mixed**: fitness courses, badminton, Shorinji Kempo (Martial art), ski players, soccer football players, swimmers, volley ball players, basket ball players and hockey players. No difference of the K content of the FFM was found between groups (table 3.16.).

By this classification it can be seen that each group has about the same value.

Whether by intensity or by type, physical activity has been shown not to be a good predictor of muscularity as proposed by Womersley et al, 1976, because the type of body is not only determined by the physical activity but also by other factors such as genetic, dietary habits and age (Shukla et al, 1973).

TYPE OF PHYSICAL ACTIVITY	T B K / F F M [mmol/kg]			
	FEMALES		MALES	
MIXED	(n=12)	64 ± 3.6 (57 - 70)	(n=11)	68 ± 3.5 (60 - 73)
STRENGTH	(n=3)	65 ± 2.7 (63 - 69)	(n=16)	69 ± 3.8 (60 - 75)
ENDURANCE	(n=8)	63 ± 3.3 (57 - 67)	(n=5)	69 ± 3.6 (63 - 71)
ALL	(n=23)	64 ± 3.4 (57 -70)	(n=32)	68.6 ± 3.5 (60 - 75)
		p = 0.4		p = 0.9

Table 3.16. Potassium content of the FFM based on assumed density of 1.1 g/cm³ of the volunteers classified by type of physical activity.

In order to give further weight to this conclusion, comparisons between the estimations of FFM and fat% using the figures of density of the FFM and the amount of K of the FFM proposed by Womersley et al, 1976, based on the intensity of physical activity performed by the subjects representing their degree of "muscularity", and the estimations obtained without taking this into account but using constant figures and allowing only a sex difference in the amount of K of the FFM (Table 3.17).

The figures for the density of the FFM [g/cm^3] and the K content of the FFM [mmol/kg] proposed by Womersley et al, are, respectively:
for young sedentary: females 1.100 and 60; males: 1.105 and 67;
for young muscular: females: 1.090 and 63; males: 1.095 and 69.

The figures to be compared against are: 1.100 g/cm^3, for the density of the FFM for both sexes and 64 mmol^K/kg$_{FFM}$ for females and 69 mmol^K/kg$_{FFM}$ for males (this study, table 3.17).

For females, it can be seen in table 3.17, that on the mean values differences of FFM and fat% were almost nil, specially using Womersley et al's figures (W's); however, using these values the dispersion values were slightly larger than using the constant figures, i.e. those proposed by this study. Both widths of the confidence intervals (CIs), for the mean difference and for all differences were just smaller using the constant figures but were better distributed using W's figures, but were practically equal.

For males, the mean FFM and fat% values by D and TBK were more similar using the constant values than using the W's figures; therefore, mean differences were greater using W's figures but their magnitude (scatter of differences) was practically the same. The 95% CIs for the mean difference using W's figures, did not include 'zero', showing that there was a bias, i.e., significant FFM overestimation and underestimation of fat% estimated by D using W's figures. The 95% CIs for all differences were very similar using either figures.

As predicted, these results reinforce the fact that there is no advantage in using different values for groups with different levels of physical activity.

	FFM [kg]		Fat%	
	This study X ± SD	Womersley X ± SD	This study X ± SD	Womersley X ± SD
Density	45.6 ± 4.35 (32.0-54.1)	47.1 ± 4.88 (31.9-56.5)	20.4 ± 4.17 (10.5-25.5)	17.9 ± 4.97 (6.58-25.5)
Potassium 64 mmol/kg	45.8 ± 5.14 (33.0-56.5)	47.1 ± 4.88 (35.2-57.4)	20.1 ± 5.67 (6.6-29.4)	17.8 ± 5.25 (5.15-28.3)
DIFFERENCE Dens - TBK	-.21 ± 2.56 (-5.7-4.9)	-.005± 2.67 (-4.6-6.2)	0.3 ± 4.49 (-8.9-9.8)	.05 ± 4.75 (-11.2-8.1)
95% CI for mean diff.	-1.3 to .82	-1.1 to 1.1	-1.5 to 2.2	-1.9 to 2.0
95% CI for all diff.	-5.3 to 4.9	-5.4 to 5.3	-8.6 to 9.3	-9.4 to 9.5

Table 3.17.A. Comparison of body composition results using the density and amount of K of the FFM figures, based on muscularity proposed by Womersley and using constant values for all subjects for females (n=26).

	FFM [kg]		Fat%	
	This study X ± SD	Womersley X ± SD	This study X ± SD	Womersley X ± SD
Density	61.4 ± 7.52 (48.1-75.2)	62.4 ± 7.85 (47.3-80.4)	10.4 ± 3.90 (4.5-21.9)	8.9 ± 4.51 (2.5-20.2)
Potassium 69mmol/kg	61.0 ± 7.91 (47.0-78.1)	61.2 ± 7.80 (48.4-78.1)	10.9 ± 6.33 (-.5-31.8)	10.6 ± 6.14 (-.5-31.8)
DIFFERENCE Dens - TBK	0.4 ± 3.1 (-5.5-8.2)	1.2 ± 3.3 (-4.0-9.7)	-.6 ± 4.34 (-11.6-8.0)	-1.6 ± 4.54 (-13.4-6.1)
95% CI for mean diff.	-.6 to 1.5	.1 to 2.2	-2.0 to 0.9	-3.1 to -.1
95% CI for all diff.	-5.8 to 6.7	-5.4 to 7.7	-9.2 to 8.1	-10.7 to 7.5

Table 3.17.B. Comparison of body composition results using the density and amount of K of the FFM figures, based on muscularity proposed by Womersley and using constant values for all subjects for males (n=38).

As a further analysis, the relationship between the amount of potassium of the FFM (K/FFM) and some anthropometric variables, presented below, were studied (Table 3.18).

The coefficients of correlation were too low and non significant in all cases. This leads to the conclusion that anthropometric variables were not related to ~~TBK~~ K/FFM.

V A R I A B L E	COEFF. OF CORRELATION r (p)	
	FEMALES (n=26)	MALES (n=38)
Frame size. Sum of 4 standardized diameters	0.18 (p = 0.4)	0.13 (p = .4)
Arm muscle area (cm^2)	0.11 (p >0.5)	0.08 (p>.05)
Sum of 3 circumferences (arm, thigh, calf)	0.04 (p >0.9)	0 (p > 0.9)
Arm muscle area as % of total arm area	0.06 (p >0.3)	0.19 (p > .6)

Table 3.18. Relationship between ~~TBK~~ K/FFM with some anthropometric variables.

1.3. Constancy of the potassium content of the FFM.

The assumption of a constant value of K of the FFM proposed by Forbes et al, 1961 is attractive because it makes the method of TBK an independent one to measuring B.C.. However, the values derived by Forbes et al, 1961 from 4 human cadavers do not coincide with the ratio values obtained in studies in which TBK and FFM have been measured in the same individuals and by different methods. The mean value for the 3 male cadavers was 66.6 mmol/kg which is not the value most commonly used of 68 mmol/kg. For females, the only cadaver analysed had a value of 72.8 mmol/kg. It has been argued that females & males have a different amount of K of the FFM; Womersley et al, 1976 calculated from a study of Forbes et al, 1968 in which it was found that the relation Ke/TBW was 5.8% lower in females than in males, a value of 65.2 mmol/kg. This reduction was not done from 72.8 mmol/kg (the female cadaver), nor from 66.6 mmol/kg (the mean of the 3 male cadavers) but from 68.1 mmol/kg, the mean value of the 4 cadavers.

There does not exist clear evidence in the literature of the actual values for the ratio TBK/FFM for each sex. There are some isolated findings such as those of lower amounts of water and higher amounts of K in males that make the relation TBK/FFM appear as being higher for males than females.

The lower ratio TBK/FFM for females has been derived from studies such as those of Forbes et al, 1968 and Talso et al, 1960 in which TBK was derived from exchangeable potassium (Ke) and FFM from total body water (TBW).

Surveyor & Hughes, 1968 have reported that there is a considerable individual variation in the results of Ke and TBK, then the error in using a constant value to get TBK from Ke should be considered.

Variations in the proportion of TBW of the FFM are quite possible; for example, in the 8 cadavers chemically analysed, it was found that the water content of the FFM may vary between 69 to 73%. Womersley, 1974 made reference to a study of Messinger & Steele, 1949 in which measurements of TBW (by the antipyrine method) and D (to predict FFM) were performed in 9 men; the water content of the FFM varied between 68 and 77% (this range might be lower due to technical errors).

The variation in any of the components of the FFM will also contribute to variations in TBW. In a study on B.C. in Mexican subjects performed by Espinosa et al, 1992 bone mineral content (BMC) was measured by dual energy X-ray absorptiometry (Lunar-DPX) and FFM was calculated by anthropometry (Durnin & Womersley, 1974) in 199 young (mean age 24 years), healthy, physically active, women and men.

It was found that the mean proportion of BMC of the FFM is about 7% higher in females (7.1 ± 0.54) than in males (6.6 ± 0.52). An increase in the proportion of mineral would concomitantly lower water & protein proportions.

Let's assume that 72.5% TBW/FFM were the mean normal value and that a higher mineral content lowers water proportion to 71%. If a female individual had 35 liters of water she would have a FFM of (35*100/72.5) 48.3 kg. If 71% is used instead (35*100/71) yields a FFM of 49.3 kg. If the amount of TBK of this individual were 3200 mmol, then 3200/48.3 = 66.3 mmol/kg and 3200/49.3 = 64.9 mmol/kg. A difference of 1.4 mmol/kg is not important, specially that it would not be a value but a range of values. This fact would not help to explain the difference of the amount of K of the FFM between sexes. The logic of this calculation could be erroneous; Widdowson, 1968 noted that the water content of the FFM in males tends to be highest in individuals who have a low bone content as estimated from the amount of calcium per kilogram FFM. The single, reasonably normal female cadaver had a high water and a high calcium content, and Widdowson suggested that women may have a higher water content in their soft tissues, particularly in their skeletal muscles, than men.

The relationship between K and TBW is a complex one. Moore et al, 1963 have shown that the higher Ke/TBW ratio in men is associated with lower ratios for extracellular water (ECW)/ TBW, Na_e /TBW and Cl_e/TBW; all these observations indicate that the extracellular fluid is relatively larger in the young female than in the male. The normal values found by these authors for the ratio ECW/TBW in young adults were ≈46% in women and ≈42% in men, but this difference become lower with increasing age until it disappears by about 85 years of age. Forbes & Amirhakimi, 1970 calculated that the ratio ICW/TBW starts being slightly lower for females, or higher for males, at an age ≈14 years and increases to reach a difference of about 5% in young adults; these authors conclude that this difference would account entirely for the observed sex difference in the ratios K_e/TBW and K_e/FFM.

There is a controversy about a sex difference in the ratio K_e/ICW. Moore et al, 1963 did not find a significant difference between sexes; but Cheek, 1968 did, and attributed this difference to a greater amount of K within the intracellular water of boys after the age of about 7 years (when the content of K in the body is about 1200 mEq). In relation to this, Womersley, 1974 pointed out that this higher ratio may have been due to increased absorption of the γ-rays by the greater amount of subcutaneous fat in the girls. Then there might not be such a difference.

Another suggestion has been that the amount of water in skeletal muscle is proportional to the FFM. Widdowson (1968) has suggested that women may have a higher water content in their soft tissues, particularly in their skeletal muscles, than men. Pitts and Bullard, 1968 found a negative correlation between the percentage of water in skeletal muscle and the FFM in non-primate mammals; these authors also showed that there was a significant decrease in the water content of the FFM with increasing weight of the FFM from about 78% in the smallest mammals to 71% in cattle ($r = -0.76$).

All these observations show how the proportion of water of the FFM could easily vary and if FFM is derived from TBW some mistakes could be introduced in the first instance. Then the generation of the ratio TBK/FFM as a mean value for each sex, generates a second chance of error.

1.4. Constancy of the density of the FFM.

The B.C. results obtained by densitometry (D) were calculated on the assumption that the density of the FFM is 1.100 g/cm^3.

To prove that the density of the FFM is constant in individuals with different B.C., is difficult; however, the evidence from the cadaver analyses makes one to believe that the density of the FFM is "fairly" constant (depending basically on the amount of mineral which has a high density compared with protein and water).

It has been found that the composition of the FFM is fairly similar between warm-blooded adult animals.

In man, the chemical analysis of 8 human cadavers (6 males and 2 females) studied by different authors (3 of them corrected by Womersley, 1974 because of obvious overhydration) has shown the following ranges of water, protein and mineral of the FFM: 69.4-73.2%, 19.2-23.8% and 6.0-7.6%, respectively. These ranges include the variability due to different analytical techniques used by each author. The water and protein content of the FFM of men appear to be lower than for animals; the reason is a greater amount of mineral because of his relatively larger skeleton.

From the anatomical dissections of 21 human cadavers (16 males and 5 females) a considerable variation was found in the composition of the "lean tissues" of the body; skeletal muscle for example comprises from little more than a third up to little more than a half, and the weight of the skeleton varied from 17% up to 23.1% of the weight of the lean tissue in the different cadavers. It is indeed very difficult to analyse precisely the composition of the body from anatomical dissections.

Behnke et al, 1942 indicated that given that the composition of the lean body mass (LBM) of man was found to be fairly constant, its specific gravity (S.G.) would be relatively fixed depending basically on the content of high S.G. bone mineral. Under the assumption that the lean body mass (LBM) has 10% of essential lipid and 5% mineral content they calculated that the S.G. of the LBM would be 1.082 units. If instead of 5% mineral, 7% is assumed, the S.G. becomes 1.095 units.

Based on the density of the anatomical constituents of the FFM, von Döbeln, 1956 calculated the density of the soft tissue component of the anatomical fat free (FF) as derived from the anatomical data on the composition of 5 human cadavers. Womersley, 1974 completed the information adding the data of 2 more cadavers. The rounded off mean values for the proportions of each tissue in anatomical FFM and the correspondent density [g/cm^3] were: muscle 48%, 1.043 g/cm^3; skeleton 21%, 1.25 to 1.30 g/cm^3; skin 8%, 1.053 g/cm^3; liver 3%, 1.059 g/cm^3; C.N.S. 3%, 1.035 g/cm^3; blood 9%, 1.052 g/cm^3; other tissues 8%, 1.043 g/cm^3. The calculation of the density of the anatomical FFM minus its bone content (dens. anat. FFM - bone) was: 1.045 g/cm^3. Some considerations regarding the sources of error in this calculation were analysed by von Döbeln; the first one was that the density of the skin, because of its low water content, might have been higher, the second was the variation in water content of the body that can make B.M. to vary $\pm$ 0.5 kg daily: such variations in water will cause the 'dens. anat. FFM - bone' to vary between: 1.043 to 1.047 g/cm^3. The third, and more important observation is a report that the density of the skeletal muscle of guinea pigs can be as high as 1.071 g/cm^3, mean 1.064 g/cm^3, (Gersh et al, 1944). Since the skeletal muscle is the largest component of the body, a change in its density value of this magnitude, would produce an important variation in the overall 'dens. anat. FFM - bone'; for example, if 1.064 g/cm^3 was used, 'the dens. anat. FFM - bone' would become 1.057 g/cm^3.

Another report on the density of FF muscle in mature white rabbits and dogs was done by Méndez and Keys, 1950. For 13 rabbit muscles the mean $\pm$ SD was 1.0609 $\pm$ 0.0011 and for 12 dog muscles 1.0620 $\pm$ 0.0021 g/cm^3. These values are near the mean value found by Gersh et al, 1944.

von Döbeln, 1956, calculated limiting values for the density of the anatomical FFM taking into account the above mentioned variations of the water content of the body, but also of bone density, and of the proportion of the skeleton of the anatomical FFM.

The density of bones may vary from 1.21 (spongy bone) to 1.96 g/cm^3 (compact bone) but the mean density of the whole human skeleton probably lays between 1.25 and 1.30 g/cm^3 for high and low fat bones,

respectively (Forbes et al, 1953). The proportion the skeleton represents of the anatomical FFM of the cadavers included for von Döbeln's calculation vary from 17.7% to 23.1%, and the corresponding soft tissues from 76.9% to 82.3%. The limiting values derived by von Döbeln are: 1.093 as the highest to 1.077 g/cm³ as the lowest. These values compare closely to those proposed by Behnke for a LBM with a 10% of essential lipid.

Womersley, 1974 calculated the density of the true FFM from von Döbeln's values, under the assumption that the anatomical FF tissue still has a content of chemical fat; using a 10% value as an average at a density of 0.9 g/cm³, the limiting values become between 1.100 & 1.120 g/cm³. Also, if the density of the skeletal muscle is taken into account and the mean value reported by Gersh et al, of 1.068 g/cm³ is used, then the limiting values become 1.077 to 1.105 g/cm³ for the anatomical FFM, and 1.100 to 1.134 g/cm³ for the true chemical FFM.

These wide ranges, Womersley says, "probably mainly reflect uncertainty in the true values for the composition and the density of the components of the FFM; differences between individuals are possibly quite small. However, there is likely to be some variation in the proportions of skeleton and soft tissue present in different individuals".

Clarys and Martin, 1985 calculated the density of the FFM of 4 cadavers analysed by Mitchell, 1945 (#1) and Forbes, 1953, 1956 (#2,3,4) regarding the FFM as a 3-component system composed of fat free (FF) muscle, FF bone and FF residual:
 # 1) 34.9, 14.1, 51 %; **# 2)** 46.1, 16.4, 37.5 %; **# 3)** 50.6, 16.2, 33.2 %; **# 4)** 40.3, 15.7, 44 %, respectively. Using the values of 1.070 g/cm³ for the density of FF muscle (Mendez & Keys, 1960 and Allen et al, 1959); 1.431 g/cm³ for FF bone (calculated from the mean bone composition of 4 human cadavers studied by Mitchell and Forbes: 18.6% fat, 32.4% water and 19.8% mineral) and 1.039 g/cm³ for FF remainder (R) (calculated using the mean values from the 4 cadavers for the composition of the FFM, i.e. FF muscle: 42.57%, FF bone: 15.80%, R: 41.63%), the densities of the FFM for each cadaver was: 1.093, 1.103, 1.104 and 1.099 g/cm³.

Clarys and Martin, 1985 made note that the mean value has been assumed to be 1.100 g/cm³ in order to calculate the density of R but that in reality, for these cadavers it may deviate substantially from this and that only the effects of variation in the amount of FF muscle and FF bone have been considered. Thus, the densities of all components including the residual, R, have been assumed to be constant. In reality, these additional sources of variation would

create a wider ranges of densities for the FFM. Table 3.19 summarizes the limiting values that have been mentioned.

The physical activity of the subjects of the present study covered a wide spectrum of intensities and types, ranging from those, very few, that performed only the necessary movements for their basic needs, to those that were completely dedicated to a given activity at an amateur or professional level; then the muscle mass% of this sample must also cover a wide spectrum, but on average, higher than for the general population.

A question that is still to be answered is: will the density of the FFM change with the concomitant, possible variations in the proportion of muscle mass? And, would the variation in the density of the FFM caused by different relative amounts of muscle mass be important in terms of the known uncertainty of the exact density for each component of the FFM?

A variation from 45 to 51% of the proportion of skeletal muscle of the lean tissue compartment ("body mass-adipose tissue mass") has been reported for the 7 human cadavers anatomically dissected, chosen by von Döbeln & Womersley. This variability was analysed in the context of the limiting values of the density of the FFM above studied to know how much will the density of the FFM change as a function of muscularity.

Using the information of the anatomical data of the 7 human cadavers and the densities of the various constituents of the body, used by von Döbeln & Womersley to calculate the limiting values for the density of the anatomical and chemical FFM, the density values of the FFM were calculated, taking into account variations of skeletal muscle and skeleton, each separately or combined and using as examples the cadavers that had the extreme values.

AUTHOR	VALUES	COMMENTS
Behnke, 1942	S.G. of LBM 1.082 to 1.095	variation due to the content of bone mineral (5-7%), assuming 10% of essential lipid.
von Döbeln, 1956 & Womersley, 1974	D of LBM 1.077 to 1.093	Data of 7 cadavers anatomically dissected, taking into account variations of water, density of whole skeleton (1.25-1.30 g/cm^3) and proportion of skeleton (17.7-23.1%).
Womersley, 1974	D of FFM 1.100 to 1.120	Same as 2, and assuming that LBM has a mean fat content of 10%.
Womersley, 1974	D of LBM 1.077 to 1.105	Same as 2, variation due to the density of skeletal muscle from 1.043 to 1.068 g/cm^3.
Womersley, 1974	D of FFM 1.100 to 1.134	Same as 4, but subtracting 10% of essential lipid to achieve FFM instead of LBM.
Womersley, 1974	D of FFM 1.101 to 1.117	from 8 human cadavers chemically analysed.
Clarys & Martin, 1985	D of FFM 1.093 to 1.104	from 4 human cadavers chemically analysed.

Table 3.19. Proposed limiting values of the specific gravity (S.G.) or density (D) for the "anatomical FFM (LBM)" or "true FFM (FFM)".

Table 3.20. shows the calculated densities of the FFM taking into account the reported variations for the proportion and for the density of skeleton and skeletal muscle. A mean common density for skin, liver, central nervous system, blood and 'other tissues' (residual) was used to make it vary as a whole entity according to the variations of the skeleton and skeletal muscle using a common density value of 1.049 g/cm³.

From this table it can be seen that variations in the proportion of skeletal muscle, the component that forms around half of the anatomical fat free tissue, produced a difference of 0.0003 to 0.001 g/cm³, depending on the value used for the density of the skeletal muscle (1.043 or 1.064 g/cm³), in the density of the FFM, (examples 1 & 2). The most important finding is that a different value attributed to its density caused a considerable discrepancy (example 3).

The variation in the proportion of the skeleton from 17.7 to 23.1%, caused a higher discrepancy than a variation in its density from 1.25 to 1.30 g/cm³ (examples 4 & 5). These limiting values are virtually the same as those derived by Behnke and by von Döbeln & Womersley.

Example 6 shows the limiting density values, using as examples the cadavers that presented the extreme values for the skeleton, i.e., 17.7 & 23.1% and using the 2 values that have been suggested for the density of the skeleton, i.e., 1.25 & 1.30 g/cm³ and the cadavers that presented the extreme values for the skeletal muscle, i.e., 44.9 & 51% and using the values for the density of skeletal muscle of 1.043 & 1.064 g/cm³, ranged between 1.077 and 1.104 g/cm³, for the anatomical FFM and between 1.098 to 1.129 g/cm³ for the true chemical FFM (assuming 10% as the content of fat in the anatomical fat free tissue). These limiting values are similar but narrower than those obtained by Womersley, 1974 (1.100 to 1.134 g/cm³) taking into account all variations but that of the proportion of the skeletal muscle. As shown in examples 1 & 2, an increase in the proportion of skeletal muscle alone, practically did not change the density of the FFM. The difference between this exercise and that performed by Womersley is mainly due to the fact that in the present one, cadaver values and not the extreme values on their own were used, to be sure that the combination of values did exist in reality.

	Skeleton	Skeletal Muscle	residual (density = 1.049 g/cm³)	density of the LBM g/cm³	density of the FFM g/cm³	difference in D FFM
	% 20.0	45.0	35.0	1.0882	1.1114	
	d 1.30	1.043				-.0005
1	to					
	% IDEM	51.0	29.0	1.0878	1.1109	
	d	1.043				
	% 20.0	45.0	35.0	1.0984	1.1231	
	d 1.30	1.064				.0011
2	to					
	% IDEM	51.0	29.0	1.0993	1.1242	
	d	1.064				
	% 21.3	49.2	29.5	1.0908	1.1144	
	d 1.30	1.043				.0129
3	to					
	% IDEM	49.2	IDEM	1.1020	1.1273	
	d	1.064				
	% 17.7	44.9	37.4	1.0832	1.1057	
	d 1.30	1.043				.0134
4	to					
	% 23.1	46.5	30.4	1.0949	1.1191	
	d 1.30	1.043				
	% 20.0	48.0	32.0	1.0808	1.1029	
	d 1.25	1.043				.0083
5	to					
	% 20.0	IDEM	32.0	1.0880	1.1112	
	d 1.30					
	% 17.7	44.9	37.4	1.0769	1.0984	
	d 1.25	1.043				.0211
6						
	% 19.1	51.4	29.5	1.095	1.1195	
	d 1.30	1.064				.0307
	% 23.1	46.5	30.4	1.1035	1.1291	
	d 1.30	1.064				
	% 16.3	59.4	24.3	1.0735	1.0946	
	d 1.25	1.043				.0427
7	to					
	% 25.7	41.9	32.4	1.1107	1.1373	
	d 1.30	1.064				

Table 3.20. Limiting values for the density of the FFM making vary the amount of skeleton, skeletal muscle and their density.

Even wider ranges than those already exposed are those from the anatomical cadaver analyses of 13 elderly, Brussels subjects (B.C.S.) of both sexes, reported by Clarys and Martin, 1985. They found a range for skeletal muscle from 41.9-59.4%, for skeleton from 16.3-25.7 and for residual from 24.0-32.4%. Example 7 shows these ranges, the limiting density values for the anatomical FFM ranged from 1.0735 to 1.1107 g/cm^3 and for the true FFM from 1.0946 to 1.1373 g/cm^3.

The limiting FFM density values showed differences of 0.031 and 0.043 g/cm^3, for the cadavers used by Womersley and for the 13 Brussels cadavers, respectively. These differences are too large but if only variations in the proportions of tissues were taken into account the differences become 0.014 and 0.021 g/cm^3, respectively.

The doubt about the true densities of the compartments of the FFM, specially that of the skeletal muscle must be clarified. In the case of the skeleton, Forbes et al, 1953, reported that the mean density values of the whole skeleton probably lay between 1.25 g/cm^3 (the density of the tibia, which has a high fat content) and 1.30 g/cm^3 (the density of the ulna which has very little fat and a relative high mineral and protein content); then, the real density value, may lay between these two values. Clarys and Martin, 1985 derived a value for the FF bone of 1.289 g/cm^3 based on the mean bone composition of the cadavers of Mitchell and Forbes, the 4 cadavers had a mean bone fat% of 18.6 and not the assumed 10% commonly used as the essential fat and used in this example to yield true FFM. In the case of the skeletal muscle, values from 1.043 to 1.064 g/cm^3 have been used in this example and this range produces an important difference in the calculation of the density of the FFM. Clarys and Martin used the value of 1.070 g/cm^3 (Méndez and Keys, 1960) but this value refers to the FF cells which is the calculation of the density of the FF tissue without any extracellular fluid which is part of the FFM and if it is excluded, it will have to be somehow included.

A practical illustration of the error that could be caused by using the maximum variation, i.e., 0.021 g/cm^3, of the calculated theoretic density of the FFM from the cadaver data of Clarys and Martin, 1985 for the estimation of B.C., would be a female with 56 kg and a measured body density of 1.050 g/cm^3. If 1.098 and 0.9 g/cm^3 were used as the densities of FFM and fat, respectively, her B.C. would be: 21.4% fat, 12 kg fat and 44 kg FFM. If 1.119 (1.096+0.021) and 0.9 g/cm^3 were used instead, the composition would be 26.1% fat, 14.6 kg fat and 41.4 kg of FFM. These differences are important but the limiting density values using the data of the cadavers chosen by Von Dölben and Womersley, had a smaller range (0.014) and these cadavers were analysed by different investigators and using different techniques; it is interesting how the cadavers reported by Clarys et

al, 1984 showed a wider range when the sample was more homogeneous: elderly and from one nationality.

The group of individuals of the present study most probably had a higher than normal proportion of muscle mass, but as it has been seen, that makes the density of the FFM to change only slightly. More important is the density value that should be attributed to it and also important is the proportion and density of the skeleton that make a more important difference.

In relation with the chemical components of the FFM, Womersley, 1974, calculated the density of the FFM from 8 human cadavers chemically analysed, based on established densities [g/cm^3] of 0.9937 for water, 1.34 for protein, and 3.04 for mineral. The mean component values of these 8 cadavers was: 72% water (69.4-73.2), 21% protein (19.2-23.8) and 7% mineral (6.0-7.6). The range for the density for the true FFM obtained was from 1.101 to 1.117 units, the mean value being 1.106 g/cm^3. In relation to this range of values, Womersley refers: "considering the variation in sex, age (25-60 yrs.), race and probable physical condition of the cadavers, and the fact that the analyses were carried out in 2 laboratories using quite different techniques, the variability is quite small".

A theoretic exercise was done to study how much variation in the overall mean FFM density of the cadavers would cause the variability that each component presented, allowing the other two components to vary complementary and using a common density.

As it can be seen from table 3.21. water variations produced the widest variability in the density of the FFM, either because of its low density or because of the effect it causes in the other two components with higher density values. At the lowest extreme of the limiting values for FFM density, is the lowest value of mineral content, i.e., 6%, and at the other end, is the lowest value of water content, i.e., 69.4%.

The calculations of the density of the FFM by anatomical cadaver analysis was 1.110 g/cm^3 and by chemical analysis 1.106 g/cm^3. This would mean that the average value for the density of the FFM might be slightly higher than 1.100 g/cm^3, but the exact value is uncertain.

Even though the obtained limiting FFM density values are not too narrow and the value for the density of the FFM might be higher than assumed and that big mistakes can be done by using an attributed density of the FFM that does not correspond with the true value, no better value than the one established, i.e., 1.100 g/cm^3, should be used until there are more studies that confirm the true densities of each tissue or chemical component, their true variation, and their relative amounts.

COMPONENT Density (d) Proportion (%)	COMMON COMPONENT Density (d) Proportion (%)	Density of the FFM g/cm³
MINERAL **d = 3.04 g/cm³**	PROTEIN & WATER d = 1.055	
6 %	94%	1.0980
7 %	93%	1.1055
7.6 %	92.4%	1.1100
PROTEIN **d = 1.34 g/cm³**	MINERAL & WATER d = 1.058	
19 %	81%	1.1021
21 %	79%	1.1069
23.8 %	76.2%	1.1138
WATER **d = 0.9937 g/cm³**	MINERAL & PROTEIN d = 1.564	
69.4 %	30.6	1.1850
72 %	28.0 ~~26.8~~	1.1067
73.2 %	26.8	1.1013

Table 3.21. Density of the FFM when mineral, protein & water get the extreme values found in 8 cadavers.

1.5. Use of a regression equation to estimate fat free mass from potassium.

The figures for the ratio K/FFM that has been found for different groups of subjects is of wide variation. Most mean values for women are around 56-60 mmol/kg and for men around 60-64 mmol/kg; but the whole range of mean values are from 48-70 mmol/kg. These values are indeed much lower than the classical value of 68 mmol/kg and it seems that each ratio is specific for the group studied (see literature review, section 7.2).

The observation that the amount of K/FFM is lower in females has been found by several authors, but the physiological phenomenon has not been explained; only the hypothesis made by Womersley et al, 1972 and supported by Delawaide & Crenier, 1973, that the difference between sexes, could be attributed to variable proportions of tissues of the FF compartment. On the other hand, there are studies that report no difference in the amount of K/FFM between sexes (Burkinshaw & Cotes, 1973 and Lye, 1981).

The present study showed variations from 57 to 72 mmol/kg for the female group and from 60 to 75 mmol/kg for the male group, that are at the upper end of the range of all the studies reviewed. Most of the K/FFM values of both sexes overlap in most part of the range of values, it is true that the lowest values are for females and the highest are for males and that mean values were always significantly different between sexes. But the wide range of ratios found within each sex shows that there are also important differences and it could be related to the composition of the FFM independently of the sex. Figures 22 & 23, App. 2, showed that there is a trend to different behaviour for subjects as their fat% changed; i.e., for leaner individuals of either sex, fat% by D was higher than K and most of the not-so-lean subjects had fat% by K values grater than D.

It seems obvious that the amount of K of the FFM is not constant for changing size, but the correct value in different instances is not known and the calculation of FFM from TBK cannot be based on the use of a ratio, even when subjects are grouped, as it was seen in the previous section. Then, the regression option was studied.

The following equations were obtained:

FEMALES (n = 26)

FFM = 11.98($\pm$ 3.96) + 0.0114 (TBK [mmol]) ; r = 0.87; RSD = 2.21 kg;

CV (TBK) = 11.005 CV (FFM) = 11.35 CV_x / CV_y = 0.97

MALES (n = 38)
FFM = 8.12(± 3.83) + 0.0127 (TBK[mmol]); r = 0.92; RSD = 3.00 kg; CV (TBK) = 12.96 CV (FFM) = 11.86 CV_x / CV_y = 1.09

For both equations the slope was different from 1 and the lines did not pass through the origin ($p< 0.05$) (figure 26, App. 2). For both sexes the CV_x/CV_y did not equal the respective r.

The females' and males' straight lines were compared to investigate whether each sex have different (K/FFM). It can be seen, from figure 26, App. 2, that it is not possible to distinguish between the female and male regression lines; they are practically one line. This, in itself, lends support to the possibility that females and males do not have different K/FFM.

Nevertheless, an statistical approach was explored to be sure that the observed line did not occurred by chance. In other words, "to be statistically precise in the comparison of the two regression lines, it is necessary to take into consideration the sampling variability of the data through the use of statistical tests" (Kleinbaum & Kupper, 1978). The questions to be answered (tested in the way proposed by Kleinbaum & Kupper, 1978) were:
1- are the two slopes the same or different (regardless of whether or not the intercepts were different) ?
2- are the two intercepts the same or different (regardless of whether or not the slopes are different) ?
3- are the two lines coincident (i.e., the same) or do they differ in slope and/or intercept ?

The test of parallelism, which compares the two slopes, did not show sufficient evidence to reject the hypothesis of parallelism: the lines for females and for males have the same slopes.

The test of intercepts, showed that there is a common intercept.

The test for coincidence from separate straight-line regression fits, showed that the lines are the same, i.e., the slopes and the intercepts are equal.

In addition to the preceding tests it was also determined whether or not the strength of the straight-line relationship was the same for both sexes. This was done by testing the equality of correlation coefficients (as proposed by Kleinbaum & Kupper, 1978), which showed equality.

The common regression line for both sexes is shown in figure 27, App. 2, and had the form:
FFM = 9.23 + 0.0124 * TBK [mmol]; r = 0.96; RSD = 2.68 kg
CV (TBK) = 21.21 CV (FFM) = 18.33 CV_x / CV_y = 1.16

The condition to use the ratio standard K/FFM stated by Tanner (1949), that the CV_x / CV_y = r, was not satisfied by the data in this study. The ratio of the CsV is 1.16, and since r can never be this large, no regression line can exist which would coincide with the ratio line and then, a regression standard would be indicated in this case. However, the FFM figures did not differ much using either the regression or the ratio standard, and the error caused by the use of the ratio standard would be small because the ratio standards used were the ones for this specific population (64 & 69 mmol/kg FFM for females and males, respectively).

The use of the ratio standard assumes that the amount of K is a constant amount of the FFM and that there is a different value between females and males. The constant amount may be derived from the mean value of K/FFM and is therefore population specific; figure 28, App. 2, shows the relation of FFM and TBK for females and for males in this study. It can be seen that the lines of the ratio standard pass through the point of the two means, and by virtue of the form of the equation, also through the origin. The regression line passes quite near the ratio standard lines, that are already population specific, but for females the assumed amount of potassium of the FFM originally employed was 60 mmol/kg (figure 29 App. 2), the ratio standard line using this figure would be further away from the regression line and the group of women of this study would appear as having higher amount of FFM, as the results of section 4 illustrated.

The fact that K/FFM [mmol/kg] is different for females than for males would have physiological transcendence. Several authors have found different values for both sexes, but the value of K/FFM varies depending on the population studied, it depends to a large extent on the scaling technique used. Two opposed conclusions can be drawn depending on the statistical analysis selected. The comparison of the ratio standards K/FFM suggests that there is a significant difference ($p< 0.001$) between women and men: 64 and 69 mmol/kg FFM, respectively; however, the detailed examination of the distribution of the data illustrated in figure 26, App. 2, suggests that the regression line of females and males is the same, i.e., there is only one population. This observation is confirmed when the regression lines are subjected to appropriate statistical analyses. The results then suggest that there are not differences in the K/FFM between sexes. As it has been seen, the condition of equality between the relation of the CsV 'x/y' with the coefficient of correlation was not reached; therefore, it is clear that in this instance, the use of the **regression** standard is the correct option because as Tanner, 1949 has pointed out "the use of **ratio** standards, although attractive, may be misleading because they misinterpret the variables under scrutiny".

Stepwise multiple regression analyses were performed to find out whether the inclusion of the following measured or obtained variables could improve the prediction of the FFM made by TBK: body mass, height, BMI, age, each skinfold, each bone diameter, each body girth and the sum of skinfolds, sum of bone diameters and sum of girths. The best equation for both sexes had the form:

FFM = -1.92 + (0.0082 * TBK[mmol]) + (0.415 * body mass)
r = 0.98; RSD = 1.99 kg

2. COMPARISON BETWEEN THE DENSITOMETRY AND THE SKINFOLDS METHODS.

2.1. Analysis of the disagreement between the methods.

The comparison of the results of B.C. between densitometry (D) and skinfolds (SkF) were not so different on the average values of this population. The mean values for D and SkF were, respectively 19.6 vs 22.0 for fat% and 44.4 vs 43.1 kg for FFM for females and 10.7 vs 12.7 for fat% and 61.4 vs 59.9 kg for FFM for males (tables 3.3.A. & B.).

Based on these results it appears that these two methods predicted B.C. with a fair agreement; however, the concordance values were very low for both sexes (R_1æ = 0.05 for fat% for both sexes, 0.23 & 0.37 for FFM and 0.015 & 0.002 for body density (B.D.), for females and males, respectively). Paired t-tests showed the differences of fat% and FFM between these two methods for both sexes, to be significantly different ($p < 0.0001$), SkF gave higher fat% (by a mean of 2.4 units for females and 2 units for males) and lower FFM values on most subjects (by a mean of 1.3 kg for females and 1.4 kg for males); these differences were not constant along the fat% and FFM values. The range of the differences for fat% and FFM were, respectively (D-SkF) from -11 to 6 units and -3 to 6 kg for females and from -11 to 4 units and -3 to 8 kg for males; the limits of agreement were, for fat% and FFM, respectively from -9 to 4 units and -2 to 5 kg for females and -8 to 4 units & -3 to 6 kg for males (tables 3.4. & 3.5.). No relationship was found between the difference and mean for fat% nor for FFM values, then subjects with low fat% and with high FFM values presented differences between methods of the same magnitude as those not so lean subjects and with more usual B.C. values.

The characteristics of most the subjects of this study were younger, more physically active, leaner and more muscular than the general population. This fact made the sample of this group to be more specific than the group from whom the SkF equations, used in

this study to calculate fat% and FFM, were derived from (Durnin & Womersley, 1974 (D&W)).

A comparison of the general characteristics of the subjects of this study and the study of D&W shows that the subjects of the present study were on average:

- taller (165 vs 163 cm for females and 180 vs 176 cm for males),
- lighter (55.4 vs 65.3 kg for females and 68.8 vs 76.1 kg for males),
- younger (96 vs 79% of the females and 92 vs 72% of the males were younger than 40 years; there were only 3 women out of 79 (4%) vs 85 out of 272 (21%) and 6 men out of 78 (8%) vs 59 out of 209 (28%) older than 39 years),
- with lower values of each skinfold (SkF), i.e. for females and males, respectively [mm]: biceps (5.2 vs 13 and 3.2 vs 6.0), triceps (12 vs 22 and 6.9 vs 11), subscapular (9.8 vs 20 and 8.9 vs 16), supra-iliac (8.6 vs 19 and 8.9 vs 19) and the sum of 4 SkF (36 vs 74 and 28 vs 52),
- with higher values of body density (B.D.) [g/cm^3] (1.054 vs 1.026 for females and 1.075 vs 1.051 for males) and
- leaner (fat% 19.6 vs 32 and 10.7 vs 21).
- more physically active (more than 75% of the subjects practiced, on a regular basis, some sport or physical activity, whether recreational or professional vs. a preponderance of moderately sedentary, middle-class men and women),
- with a lean and lean-muscular body appearance (most of the subjects were deliberately chosen to represent lean and if possible muscular body type vs a variety of body types).

The range of values or physical characteristics of each of the variables of this study were included within the range of values or characteristics of D&W's study. The exception, but maybe not important, was B.M. for females that ranged from 40.2 to 68.2 kg vs 42.3 to 85.2 kg.

The relationship between D and SkF is different in this group of subjects to that for the group studied by D&W, then as a first step, it was decided to develop population specific equations, i.e., for young females and males, physically active, with lean-muscular body type.

Stepwise multiple regression analyses to estimate B.D. were performed, sex, age and all the anthropometric variables including B.M., height, each skinfold, its logarithm and all possible combinations of sums, each of the 3 body girths and its sum, each of the 4 bone diameters and its sum.

Sex was chosen as the first important variable, in the relationship between SkF measurements and B.D. (F value = 150; $p<0.0001$). Figure 30, App. 2, shows that a given Σ4SkF [mm] corresponds to a lower value of B.D. [g/cm³] in females than in males.

The rest of all variables were included for stepwise multiple regression analyses for each sex separately. The best single equation which got the lowest significant error of the estimate for each sex, were: Body density [g/cm³] =

Females (n = 78):

1.1462 - 0.0599 * log Σ4SkF [mm]; r = 0.71; RSD = 0.0073

Males (n = 78):

1.1564 - 0.0517 * log Σ4SkF [mm] - 2.98 * 10^{-4} * age [years]; r = 0.69; RSD = 0.0065

By multiple regression analyses the incorporation of height, B.M., body girths and bone diameters did not significantly reduced the RSD.

It was interesting to note that the logarithm of the Σ4SkF was chosen for both sexes. The log. transformation had been previously performed to the regression analysis because of the knowledge that the relationship between D and SkF may not be rectilinear because of a larger proportion of the body fat which is situated subcutaneously with increasing obesity. Figure 31, App. 2, shows that for this set of values there is not an exception and that the log. transformation made the relationship to be linear.

Age was chosen by the stepwise analyses just for males (F value = 10.6), improving the r from 0.64 to 0.69 and reducing the RSD from 0.0069 to 0.0065 [g/cm³], related to the equation in which the logarithm of the Σ4SkF was the unique variable.

Age and sex have been found to be significantly related to the estimation of B.D. from SkF thickness by D&W in their study of 481 men and women in the age range from 16 to 72 years; they found that the value for B.D. which corresponds to a given Σ4SkF decreases by about 0.004 and 0.005 g/cm³ per decade for females and males, respectively.

Forbes and Amirhakimi, 1970 in a study of 472 boys and girls aged between 7½ to 18 years also noted that age was related to the estimation of body fat from measurements of ^{40}K and skinfolds.

In the present study age was found not to be significantly important to predict B.D. from SkF thickness in females, whereas it

was for males; therefore it was decided to study this relationship further.

In order to compare the results of this study to those of D&W, subjects were grouped by age: less than 20, 20-30, 30 to 40 and 40 years and over. Analyses of variance of B.D. on the logarithm of the Σ4SkF (Log-Σ4SkF) by age groups, showed the following results: an F value = 1.35; p = 0.27 for females and F = 3.23 p = 0.03 for males. It could be noted that the F value for males diminished from a F = 10.7; p = 0.002 for the whole group to a F = 3.23; p = 0.03, for the subjects grouped by age but age was still significant; for females the results remained almost equal. This fact made analyse the individual Σ4SkF and B.D. values of those subjects at the greatest extremes of the age range.

For males, it was found a subject with 53 years of age with a Σ4SkF = 27 mm and a B.D. = 1.053 g/cm^3; other 5 males with the same value of Σ4SkF had a mean B.D. = 1.079 g/cm^3 and their age ranged from 20-32 years.

age[yrs.]	body density g/cm^3
20	1.084
23	1.075
24	1.080
30	1.080
32	1.072
53	1.053

It is obvious that this lean 53 years old man had a B.D. much lower than his younger counterparts.

The following eldest man was 52 yrs. old with a Σ4SkF = 52 mm and a B.D. = 1.049 g/cm^3. Another man with a Σ4SkF = 51 mm, had a B.D. = 1.067 g/cm^3 and 18 years old and another subject with a Σ4SkF = 61 had a B.D. = 1.056 g/cm^3 and 31 years old. From these values it can be seen that this man also had, comparatively with the other 2 younger men, a low B.D..

The following eldest man, had 47 years old; from this age downwards there was found that a given Σ4SkF corresponded to a large range of values of B.D. but age was not noticed to be particularly different. Following are the values for men older than 35 years:

age [yrs.]	body density [g/cm³]	Σ4SF [mm]
47	1.077	28
44	1.075	21
44	1.087	26
40	1.074	18
39	1.076	22
38	1.077	22

The eldest women had 63 years old, a Σ4SkF = 29 mm and a density = 1.051 g/cm³. Here is data of some other women with the same value of Σ4SkF:

age [yrs.]	body density [g/cm³]
17	1.066
26	1.058
32	1.071
32	1.054
38	1.045
63	1.051

The 63 years old woman was not the one with the lowest B.D., may be as it could have been expected. More noticeable was the 32 years old woman with the highest B.D. of 1.071 g/cm³, a value more near to those of men. Those women of over 35 years had the following values:

age [yrs.]	body density [g/cm³]	Σ4SF [mm]
37	1.058	54
38	1.056	35
38	1.045	29
41	1.036	51
45	1.055	28
63	1.056	29

It calls the attention that the 2 eldest women had about the same values of B.D. and Σ4SkF and that a younger woman of 38 years with practically the same Σ4SkF with a much lower B.D. value and another 38 years old woman with a B.D. value similar to the eldest

woman but with a higher Σ4SkF. For this group of females, it is confirmed that age was not an important variable in the relationship between B.D. and SkF.

An analysis of variance was performed for the regression of density on log-Σ4SkF and age, leaving out the 2 men older than 50 years. The F value was = 2.6; p = 0.11. The regression analysis including age had a RSD = 0.00628 and without including it = 0.00634. Then for men younger than 50 years age did not significantly reduced the RSD and therefore it is not worth including it for the prediction of B.D. from SkF.

Since the equations of D&W have reached an extraordinary success in most practical situations for nutritionists and clinical workers wishing to assess B.C., it was deemed adequate to keep the same scheme as they presented their results, so as to give a continuity to this work.

Linear regression equations were formulated to estimate B.D. from single SkF measurements, and from the combination of the sums of two, three and four SkF. Age was not considered in the equations because as it has just been demonstrated age was not justifiable to be included.

Table 3.22.A. & B. give, for females and males, all the linear regression equations for the estimation of B.D. from the logarithm of SkF thicknesses of one, and the different possible combinations of the sum of two, three and four SkF, the RSD of D and the correlation coefficients (r) in the respective linear regression equations.

The correlation coefficients (r) varied from 0.55 to 0.71 for females and 0.41 to 0.64 for males, all of them were significant at a p value < 0.001. The lowest values corresponded to the r for single SkFs; no value lower than 0.63 for females and 0.47 for males was found for sums of two and more SkF.

The use of r may not be adequate for this study as it entails the assumption that the population approximately follows the bivariate normal distribution (Colton, 1974). The sample of this study was not from a random population but was deliberately chosen to represent lean-muscular subjects and there is a preponderance of young, physically active University Students, professionals and staff. However, for purposes of comparison with other works it was convenient to count with it.

Log Skinfold (x)	Equation. Density =	RSD	r
Biceps	1.080 - 0.0348 (x)	.0083	-.59
Triceps	1.1115 - 0.054 (x)	.0077	-.67
Subscapular	1.0996 - 0.0466 (x)	.0086	-.55
Supra-iliac	1.0855 - 0.0348 (x)	.0085	-.57
Biceps + Triceps	1.1219 - 0.0558 (x)	.0074	-.70
Biceps + Subscapular	1.1156 - 0.0530 (x)	.0080	-.64
Biceps + Supra-iliac	1.1037 - 0.0445 (x)	.0078	-.65
Triceps + Subscapular	1.1344 - 0.0606 (x)	.0076	-.68
Triceps + Supra-iliac	1.1256 - 0.0552 (x)	.0074	-.70
Subscapular + Supra-iliac	1.1161 - 0.0497 (x)	.0080	-.63
Biceps+Triceps+ Subscapular	1.1404 - 0.0610 (x)	.0074	-.70
Biceps+Triceps+ Supra-iliac	1.1328 - 0.0565 (x)	.0072	-.71
Biceps + Subscapular + Supra-iliac	1.1266 - 0.0535 (x)	.0077	-.67
Triceps + Subscapular + Supra-iliac	1.1411 - 0.0593 (x)	.0074	-.69
Biceps + Triceps + Subscapular + Supra-iliac	1.1462 - 0.0599 (x)	.0073	-.71

Table 3.22.A. Linear regression equations for the estimation of body density [g/cm^3] from the logarithm of the skinfold thickness for females (n = 78).

Log Skinfold (x)	Equation. Density =	RSD	r
Biceps	1.0919 - ~~0.3396~~ 0.03396(x)	.0073	-.43
Triceps	1.1047 - 0.03606(x)	.0067	-.57
Subscapular	1.1108 - 0.0382(x)	.0074	-.41
Supra-iliac	1.0957 - 0.0225(x)	.0072	-.48
Biceps + Triceps	1.1190 - 0.0443(x)	.0065	-.60
Biceps + Subscapular	1.1260 - 0.0475(x)	.0072	-.47
Biceps + Supra-iliac	1.1119 - 0.0347(x)	.0066	-.58
Triceps + Subscapular	1.1338 - 0.0495(x)	.0067	-.57
Triceps + Supra-iliac	1.1203 - 0.03843(x)	.0063	-.63
Subscapular + Supra-iliac	1.1242 - 0.0399(x)	.0068	-.56
Biceps+Triceps+ Subscapular	1.1420 - 0.0528(x)	.0066	-.58
Biceps+Triceps+ Supra-iliac	1.1292 - 0.043(x)	.0063	-.64
Biceps + Subscapular + Supra-iliac	1.1339 - 0.045(x)	.0066	-.58
Triceps + Subscapular + Supra-iliac	1.1389 - 0.0464(x)	.0064	-.62
Biceps + Triceps + Subscapular + Supra-iliac	1.1454 - 0.0492(x)	.0063	-.63

Table 3.22.B. Linear regression equations for the estimation of body density [g/cm^3] from the logarithm of the skinfold thickness for males (n = 76; younger than 50 years).

The RSD of B.D. using the logarithm of each of the four separate SkF were greater than for the combinations of two and more SkF and ranged from 0.0077 to 0.0086 for females and from 0.0067 to 0.0074 for males; for both sexes, the smallest error were results of using the triceps (t) and the greatest from the subscapular (ss) SkFs. For the combinations of two and more SkF, the RSD ranged from 0.0072 to 0.0080 for females and from 0.0063 to 0.0072 for males; it was noticed that for males, the RSD were lower than for females and slightly lower than including age in the equations (table 3.22).

Analysis of variance studying the intensity of physical activity on the regression of D on SkF showed no significant effect (F value = 1.89; p = 0.14 for females and F = 0.87; p = 0.46 for males).

The concordance (R^2) between B.D. measured by D and B.D. predicted by SkF, using the new equations of the Σ4SkF, increased for females and males, respectively, from 0.015 & 0.002 to 0.71 & 0.66.

The selected sites to measure SkF, biceps (b), triceps (t), subscapular (ss) and suprailiac (si), were those proved at the laboratory of Prof. Durnin to give a good prediction of B.D..

The RSDs were highest for single sites but from combinations of two or more SkF lower RSD were found.

In the females the logarithm of the sum of "b + t + si" SkFs was associated with the lowest RSD (0.0072), then followed: the Σ4SkF (RSD = 0.0073) and then with the same RSD (0.0074) the "b + t", the "t + si", the "b + t + ss" and the "t +ss + si".

For males, the corresponding order was: with the same RSD (0.0063); the "t + si", the "b + t + si" and the Σ4SkF; then the "t + ss + si" (SEE = 0.0064) and the "b + t" sites (RSD = 0.0065).

For females the Σ3SkF did better than the Σ4SkF and for males a combination of sum of 2, 3 and 4 SkFs did equally as good. For both sexes the combination of "b + t + si" was the best combination but, in fact the RSDs for all the above combinations were about the same. The use of more sites should be preferred as to diminish the possible error in the measurement of a given SkF.

The mean lowest RSD found by D&W were, for females the "b + t + ss" (RSD = 0.010) and for males, the Σ4SkF (RSD = 0.0084) and for both sexes, the combination of "t + ss" (RSD = 0.010 and 0.0082 for females and males, respectively). The RSD values found for this study were generally lower than those found by D&W. The best combination of SkF vary between this study and the one of D&W; however, the RSD between the first best 5 to 7 sites of measurement were virtually the same.

Log Skinfold (x)	Equation. Density =	RSD	r
Biceps	1.1036 - 0.395(x) - .00036(age)	.0075	-.56
Triceps	1.1162 - 0.0382(x) - .00396(age)	.0070	-.63
Subscapular	1.1197 - 0.0426(x) - .000197(age)	.0076	-.53
Supra-iliac	1.1066 - 0.0253(x) - .00034(age)	.0073	-.59
Biceps + Triceps	1.1320 - 0.0476(x) - .00039(age)	.0067	-.66
Biceps + Subscapular	1.1355 - 0.0514(x) - .00022(age)	.0073	-.58
Biceps + Supra-iliac	1.1234 - 0.0377(x) - .00034(age)	.0067	-.66
Triceps + Subscapular	1.1437 - 0.0523(x) - .00027(age)	.0069	-.64
Triceps + Supra-iliac	1.1322 - 0.04101(x) - .00036(age)	.0065	-.69
Subscapular + Supra-iliac	1.1338 - 0.0424(x) - .00026(age)	.0069	-.64
Biceps+Triceps+ Subscapular	1.1528 - 0.0559(x) - .00028(age)	.0068	-.65
Biceps+Triceps+ Supra-iliac	1.1417 - 0.0458(x) - .00036(age)	.0064	-.71
Biceps + Subscapular + Supra-iliac	1.1438 - 0.0476(x) - .00027(age)	.0067	-.66
Triceps + Subscapular + Supra-iliac	1.1495 - 0.0488(x) - .00029(age)	.0065	-.69
Biceps + Triceps + Subscapular + Supra-iliac	1.1564 - 0.0517(x) - .00030(age)	.0065	-.69

Table 3.23. Linear regression equations for the estimation of body density from the logarithm of the skinfold thickness and age for males (n = 78).

Figure 30, App. 2, showed that a given Σ4SkF [mm] corresponded to a considerably lower value of B.D. in females than in males. This fact has been explained by D&W either by a greater body fat% situated internally in the females, or else that the density of the FFM is greater in males than in females.

For females, it was found that age did not produce any important influence on the prediction of B.D.. One possible reason for this, might be that the age range of the women included in the present study were younger and more physically active than the ones from the study of D&W and then the relation between subcutaneous and internal fat did not change in the age range studied. First because, as some studies have demonstrated, women up to about the age between 45-50 years the ratio subcutaneous fat:total body fat does not apparently change but for older women the proportion in the subcutaneous tissues become relatively decreased (Skerlj, Brozek & Hunt, 1953 and Young et al, 1963); and second probably because of the physical activity performed by these women. The only woman older than 45 years, did not have different B.D. than the rest of the group.

In an attempt to explain the reason for the effect of age and sex, D&W and Womersley, 1974 did a review of the studies that treat upon on the proportion of body fat situated subcutaneously and found that there is a lot of confusion about the actual values and that this fact is often overlooked even by workers on the field. These authors also reviewed the available information on SkF compressibility and deduced that this did not apparently explained the altered relationships found between D and SkF because of age but it might explain some of the differences between sexes.

Another possible source of explanation could be, from these authors point of view, that the density of the FFM may alter and that the most likely source of explanation would be the skeleton, in their review they found that there is a decrease in the mineral content of the body from 45-50 years onwards, the decrease being higher in females than in males. Using the mean rates of demineralization they calculated the possible shift in the density of the FFM and concluded that in males, the change in mineral content would not account for the difference in the position of the regression lines between D and SkF due to aging; for females, the maximum decrease that has been reported would just about explain the different positions of the regression lines.

In a study made in Mexico in 1992 (Espinosa et al, unpublished observations) on 70 females aged between 16 to 44 years and in 128 males aged between 16 to 42 years, with characteristics of B.C. and

with types and intensities of physical activities similar to those of the present study, measurements of the bone mineral mass (BMM) using a DEXA instrument (Lunar-DPX) were performed. The regression of the BMM on age showed an F value = 0.63; p = 0.43 for females and F = 0.77; p = 0.38 for males. Age explained less than 1% of the BMM. In another group of subjects, from the same sort of sample as the just mentioned study, composed of 43 females and 57 males aged between 17 to 41 years, measurements of BMM were also performed but with another DEXA instrument (Hologic QDR); the regression of BMM on age showed an F value = 4.31; p = 0.044 for females and F = 0.31; p = 0.58 for males; age explaining 9.5% and 0.5% of the BMM in females and males, respectively. However, age was also significantly related to height, older women were shorter and height was also the variable that best explained the BMM. When height was also considered in the regression analysis, the ANOVA results showed age to be no further significantly important (F value = 2.59; p = 0.116), therefore age was not related to BMM. A third sample included older subjects and with physical activity characteristics more similar of the general population (sedentary and performing light physical activities) the results for 54 females aged from 16 - 62 years were F value = 28.3; p < 0.0001, age explaining 35.2 % of the BMC; for 24 males from 15 -67 years the F value = 0.17; p = 0.69. Age and FFM were significantly related for the female group (F = 0.51; p = .02), when FFM was also included in the regression analysis, the ANOVA results showed age to be still significant with an F value = 21.1; p < 0.0001. Then for this group of females age did have a significant effect on BMM decrease.

These results show that age did not have any significant effect on BMM in males, along the age range studied (15-67 years) and in females just for the younger and physically active groups. Physical activity seems to preserve the lose of BMM with aging up to the age of 45 about years, but only a longitudinal trial, including older subjects, would demonstrate this. In those women with a wider age range and that did not exercise regularly aging was found to be significantly related to bone demineralization.

Bone demineralization has been reported by several investigators, specially for females. The use of exercise in avoiding or diminishing this effect is on debate because there has not been a definitive demonstration of an effect on bone. Exercise in experimental animals can preserve both the density and bio-mechanical integrity of bone (Barengolts et al, 1993 and Yeh et al, 1993). In contrast, there has been difficulty in demonstrating that effects of exercise in humans, particularly in longitudinal trials. Cross-sectional studies show that subjects who exercise regularly maintain

higher amount of mineral per cm^2 of surface bone; even walking is associated with a decrease of bone loss in the elderly (Krall and Dawson-Hughes, 1993). However, these results may be a consequence of subject selection (i.e., healthier people tend to exercise more). Prospective trials have generally shown that even moderate exercise does not reduce the rate of bone loss. A review article, in fact, suggests that physical activity may be an "exercise in futility" (Forwood and Burr, 1993). However, there might be some effects on the axial skeleton that are not evident in the peripheral skeleton. Aerobic training has shown to inhibit spinal, but not peripheral, bone loss (Martin and Notelovitz, 1993). Most studies, however, show no effects of even heavy, weight loading exercise, and lower levels of activity, like walking, have no demonstrable effect (Cavanaugh and Cann, 1988).

In the present investigation, age did not have a significant effect on the relation between B.D. and SkF in females and a doubtful one in males. However, age effect was found to be greater in males than in females in this and in the study of D&W. Sex effect is however, obvious. One of the possible reasons for the age and sex influence on the relation between B.D. and SkF thickness could be a shift in the density of the FFM due to the mineral content.

On the basis of the results of the above mentioned study, for the subjects of this study it would not be expected mineral to have diminished for the effect of age. First because of the age range of the subjects, second because, although doubtful, there could be some protective effect of physical activity to preserve bone mineral and third males, that showed the highest effect of age, do not seem to lose mineral even at ages greater than 45 years; then demineralization do not appear to be a cause for a different density of the FFM.

With respect to the effect of sex on the relation between B.D. and SkF thickness, based on the values from the chemical analysis of 8 human cadavers, the density of body mineral = 3.04 g/cm^3 and the combined density of protein and water = 1.055 g/cm^3, a calculation of the density of the FFM was performed using the equation:

$$Density\text{-}of\text{-}FFM = \frac{1}{\frac{fFFST}{1.0546} + \frac{fMIN}{3.04}}$$

where:

f MIN = fraction of mineral mass as a proportion of the FFM (calculated using the equations of D&W).
f FFST = fraction of fat free soft tissue (1- fraction of mineral mass).

This equation was applied to each subject of the first sample of Mexicans above mentioned, the mean ± S.D. of mineral as proportion of FFM for females = 7.1 ± .54 and for males 6.6 ± .52. The density of the FFM for the 71 females = 1.106 ± .0041 g/cm³ and for the 128 males = 1.102 ± .00392 g/cm³.

The mean proportion of the bone mineral of the FFM (BMC/FFM) was greater for females than for males and so was the density of the FFM.

Further values and discussion about the density of the FFM taking into account variations in the proportion of the BMC/FFM have been presented in table 3.19.. But differences between sexes had not been presented.

Another possibility for a change in the density of the FFM would be that greater obesity in older people may be an important factor because an increase in fat mass would imply an increase in adipose tissue that would produce a fall in the density of the FFM but, even if only the accumulation of adipose tissue were the unique cause of a shift in the density of the FFM, in the calculations of D&W this fact did not explain either the different positions of the regression lines. Their conclusion was that besides a possible variation in the density of the FFM due to the demineralization and increase of fat with aging, there must also be an important change in the proportion of body fat which is situated subcutaneously.

If the different position of the regression lines, seen in figure 30, App.2, between females and males were due to a different density of the FFM and if the density of the FFM were indeed higher for females, then the lines would have be inverted, i.e., the line for females above the line for males.

The only reason to believe that the density of FFM between sexes could be different would be by the fact that, usually males have greater amounts of muscle mass and this could diminish the density of FFM in males. But, this would be dependant on the amount of muscle and as there could be a concomitant increase in the amount of mineral, it is difficult to give a fair quantitative estimate of these shifts. Besides, in order to explain the difference between

sexes because of a different density of the FFM it would have to be greater for males.

Another reason to be considered for the different relationship between sexes between B.D. and SkF thickness, could be that there are different proportions in the ratio external (subcutaneous) : internal fat. This is congruent with the information about the variable distribution of external : internal fat. But there is no physiological reason that could explain why either sex should have a different amount of internal fat.

A new hypothesis could be that subcutaneous fat is not being accurately measured with the 4 SkF used. If the SkF sites chosen for measuring subcutaneous fat were closer to the total subcutaneous fat mass, the difference between sexes should be lower.

The sum of the four sites selected of SkF measurement: biceps (b), triceps (t), subscapular (ss) and suprailiac (si) showed to be 1.29 times greater in females in the present study and 1.42 times grater in the study of D&W, 1974. Individual SkF relations between sexes shows that b and t present greater differences between sexes and for ss and si the differences become lower. Women had greater values than males on most SkF; the values for the present study and for the D&W study were, respectively: for b 1.6 vs 2.1; for t 1.7 vs 2.0 for ss 1.1 vs 1.3 and for si 0.97 vs 1.03 times greater in females. As it can be seen, the values for the present study were lower than for the D&W study and in both studies the ss & the si SkFs presented almost no difference between sexes, specially for this study. Whereas women had only about 1.02 times greater B.D. than men in both studies. Therefore the measurement of B.D. indicates that the times women give greater values than men is a constant for both studies when estimated by B.D., but SkF shows that the times women give grater values than men is lower in the present study. This would mean that these lean women have less fat deposited subcutaneously than men, but the regression lines in figure 30, App. 2, would be explained just the opposite and there is no reason why it must be different between sexes. More probably it could be that the selected 4 SkF sites are not measuring accurately subcutaneous fat in these lean subjects in part because the distribution of fat could be different.

None of the four SkF employed measures the large storage fat of the buttocks and trochanteric areas that could be measured by combination of SkFs measured at the lower limb. Omission of these sites results in under-estimation of the subcutaneous fat, specially in women who anatomically deposit more fat in these area. It could be guessed that if SkF measurements in the lower extremity were assessed, there would be larger differences between sexes that could

account more for the total body fat mass and then, there would not be such a difference in the position of the lines in the relationship between B.D. and SkF between sexes. More or equal amount of fat in lean women, but not less, would be expected to be situated subcutaneously in women.

It should be mentioned that considerable difficulty in the measurement of SkF at the thigh area can be found, the lifting of the two fold of adipose tissue trying to separate it from the muscle is not possible and is sometimes even harmful for the subject, and big mistakes can be done. Besides, the thigh area is not easily located. Two of the characteristics for selecting SkF to measure subcutaneous adipose tissue would not be met when thigh area were included. Then, the deduction of 'body fat from those SkF possible to be measured with little possibility of error and convenient for the subject, is the best approach. Then, the portion of subcutaneous fat possible to be measured with SkF can be related to total body fat, in this case through B.D.. But accuracy must be determined and considered.

Then, besides differences in the distribution of body fat between sexes, the SkF sites selected to measure subcutaneous fat also play an important role in the different position of the regression lines of B.D. on the Σ4SkF between sexes and the overestimation of fat of the subjects of the present study using the equations of D&W may show that there is more internal than external fat in these lean subjects, these reasons also help to explain why the relationship between SkF becomes narrower between sexes.

2.2. Theoretical calculations of the changes of the chemical composition of the fat free mass related to the amount of fat and muscle in the body.

An obvious difference in B.C. between subjects of the present study and those from the study of Durnin and Womersley, 1974 (D&W) was found. If mean values of each sex from both studies were taken, theoretic calculations of the B.C. changes can be done, assuming that the mean values correspond to the data of a female and a male that have changed their B.C.

The objective of these theoretical calculations were to study the chemical composition of the body mass (B.M.) lost by exercising (and maybe also by dieting) by two subjects, that are represented by the mean values of females and males of the studies of D&W (general population) and the present one (lean-muscular population). It will also be possible to find out whether this shift in B.C. produces a concomitant change in the density of the FFM. If a density of the FFM

different to 1.100 g/cm³ were found, this would help to explain, at least in part, the reason for a different relationship between body density (B.D.) and SkF for lean-muscular subjects compared to subjects with more usual B.C.

A. Females

Mean B.M. for the D&W females was 65.3 kg with a mean fat of 32% which yields 20.9 kg fat and 44.4 kg of FFM. Mean height was 163 cm.

Mean B.M. for the females of the present study was 55.4 kg with a mean fat of 19.6% which yields 10.85 kg of fat and 44.5 kg of FFM. Mean height was 165 cm.

A correction for height was done to compensate for the 2 cm higher of the females of the present study; this correction was done by dividing the FFM by the mean height of the D&W study, ie, 44.4/163 [kg/cm] = 0.27 and multiplying it by the 2 cm difference ie, 0.27*2 = 0.54 kg and adding this amount to the FFM and B.M., ie, 44.4+0.54 = 44.9 kg FFM and 65.3+0.54 = 65.8 kg B.M..

This hypothetical woman lost 10.4 kg B.M. (65.8 - 55.4). What chemical composition could have have that mass?. There are 10 kg of fat mass lost but fat is not lost as such but as adipose tissue which is composed of about 80% triglyceride, 19% water, 1% protein and a small amount of dissolved minerals (Garrow, 1974). This means that 12.5 kg of adipose tissue were lost composed of 10 kg fat, 2.38 kg water and 0.12 kg protein.

If the lost adipose tissue is subtracted from the original mass, ie, 65.8-12.5, there would remain 53.3 kg. The actual mass is, however 55.4 kg; this means that there were 2.1 kg gained somehow in the FFM compartment. As physical activity is the main cause of the lost B.M., it is highly probable that the gained weight is mainly muscle mass, which is composed of about 80% water and 20% protein (Brozek et al, 1963). Espinosa et al, 1992 found an increase in the amount of mineral of the FFM of 0.17% between sedentary and fairly active women.

If the proportions of the FFM components proposed by Brozek et al, 1963 based on the cadavers chemically analysed were used, ie, 73.5% water, 19.6% protein and 6.9% mineral, this woman with 44.9 kg of FFM had (44.9*0.069) 3.1 kg of mineral and now she has 44.5 kg of FFM, then the proportion for mineral will be (6.9+0.17) = 7.07% therefore she has 44.5*0.707 = 3.15 kg. There has thus be gained 3.15-3.1 = 0.05 kg of mineral. The gained 2.1 kg would then now be 2.1-0.05 = 2.05 kg of muscle mass that would be composed of 1.64 kg water (2.05*0.80) and 0.41 kg of protein (2.05*0.20).

The balance would be as follows: 2.38 kg of water were lost with the adipose tissue and 1.64 kg were gained with the muscle mass. Then there was a net lose of 0.74 kg of water. 0.12 kg protein were lost with the adipose tissue and 0.41 kg were gained with the muscle mass a net increase of 0.29 kg protein was gained; and 0.05 kg of mineral were gained.

The original FFM for this female was 44.9 kg; her FFM would be composed of 33.0 kg water, 8.8 kg protein and 3.1 kg mineral. A decrease of 0.74 kg water yields 32.26 kg and an increase of 0.29 kg protein yields 9.09 kg. And an increase of 0.05 kg mineral yielded 3.15 kg. If these figures are added up 44.5 kg FFM is obtained which is the FFM obtained after losing 10 kg of B.M.. Now the relative composition of the FFM becomes: 72.5% water, 20.4% protein and 7.1% mineral.

Given a density of 3.038 g/cm^3, 1.340 g/cm^3 and 0.993 g/cm^3 for minerals, protein and water at 37°C respectively, the density of the FFM can be calculated with the aforementioned relative amounts and densities yielding a value of 1.1041 g/cm^3. This change in the relative amounts of the FFM produced a difference of 0.0041 g/cm^3 in the density of the FFM.

B. Males

Mean B.M. for the D&W males was 76.1 kg with a mean fat of 21% which yields 15.98 kg fat and 60.1 kg of FFM. Mean height was 176 cm.

Mean B.M. for the males of the present study was 68.8 kg with a mean fat of 10.7% which yields 7.36 kg of fat and 61.4 kg of FFM. Mean height was 180 cm.

A correction for height was done to compensate for the 4 cm higher for the males of the present study; this correction was done by dividing the FFM by the mean height of the D&W study, ie, 60.1/176 [kg/cm] = 0.34 and multiplying it by the 4 cm difference ie, 0.34*4 = 1.37 kg and adding this amount to the FFM and B.M., ie, 60.1+1.37 = 61.5 kg FFM and 76.1+1.37 = 77.5 kg B.M..

This hypothetical man lost 8.7 kg B.M. (77.5-68.8). This mass could have been composed as follows: there are 8.6 kg of fat mass lost; which corresponds to 10.75 kg of adipose tissue composed of about 80% triglyceride, 19% water and 1% protein (Garrow, 1974); then the composition of the adipose tissue was 8.6 kg fat, 2.04 kg water and 0.11 kg protein.

If the lost adipose tissue is subtracted from the original mass, ie, 77.5-10.75 = 66.75 kg. The actual mass is, however 68.8 kg; this means that there were 2.05 kg gained somehow in the FFM compartment. As physical activity is the main cause of the lost B.M., it is highly probable that the gained weight is mainly muscle mass,

which is composed of about 80% water and 20% protein (Brozek et al, 1963). No trend to a higher amount of the mineral of the FFM as the intensity of physical activity increased was found, as in women (Espinosa et al, 1992).

The gained 2.05 kg were basically muscle mass, composed of 1.64 kg water (2.05 * 0.80) and 0.41 kg of protein (2.05*0.20).

The balance would be as follows: 2.04 kg of water were lost with the adipose tissue and 1.64 kg were gained with the muscle mass. Then, there was a net lose of 0.4 kg of water. 0.11 kg protein were lost with the adipose tissue and 0.41 kg were gained with the muscle mass, then there was a net increase of 0.3 kg protein.

The mean FFM for the males studied by D&W was 61.5 kg. If the proportions of the FFM components proposed by Brozek et al, 1963 aforementioned are used, i.e., 73.5% water, 19.6% protein and 6.9% mineral, then the FFM would be composed of 45.21 kg water, 12.05 kg protein and 4.24 kg mineral. A decrease of 0.4 kg water yields 44.81 kg and an increase of 0.3 kg protein yields 12.35 kg. And the same 5.35 kg of mineral. The sum of these figures yields 61.4 kg FFM which is the FFM obtained after losing 8.6 kg of B.M.. Now, the relative composition of the FFM becomes: 73.0% water, 20.1% protein and 6.9% mineral.

Given a density of 3.038 g/cm^3, 1.340 g/cm^3 and 0.993 g/cm^3 for minerals, protein and water at 37°C respectively, the density of the FFM can be calculated with the aforementioned relative amounts and densities yielding a value of 1.1015 g/cm^3.

This change in the relative amounts of the FFM produced a difference of 0.0015 g/cm^3 in the density of the FFM for males.

It might be possible then, that FFM overestimation (of 0.7 kg for females and 0.5 for males) and fat% underestimation (of 1.3 units for females and 0.7 units for males) is being made by the densitometry (D) method when using a density of the FFM of 1.100 g/cm^3, as the results of these theoretical calculations show that higher values should be used for this lean-muscular population. For females a value of 1.104 g/cm^3 and for males 1.102 g/cm^3.

It is interesting that the estimation of B.D. by SkF was systematically lower than that measured by D, thus obtaining lower FFM and higher fat% than D, even using the same density of the FFM, i.e. 1.100 g/cm^3 for the calculations of B.C. Because SkF are related to B.D. to predict B.C. it could be thought that the SkF method was giving the wrong estimate of it, specially because D has been considered as the method which measurements should be compared against (reference, gold standard); however, what seems to have changed here is the density of the FFM and then the relation between B.D. and SkF also had to change but then the fact is that the basic

assumptions of the D method have changed but SkF measure more directly (external) fat than D, the potassium (K) and other methods do. The relationship between internal to external fat could also have changed as the difference of the FFM and fat% found by using a density of the FFM value of 1.100 g/cm^3 instead of the higher values herein calculated, does not fully explained the original difference found between D and SkF, but it would not be possible to quantify it, because only a theoretic analysis has been performed. Anyway, there is a lot in favour about the use of SkF to estimate body composition.

APPENDIX 1

UNIVERSITY OF GLASGOW

INSTITUTE OF PHYSIOLOGY
5th. floor, West Medical Building
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BODY COMPOSITION AND ENERGY EXPENDITURE IN LEAN MUSCULAR SUBJECTS

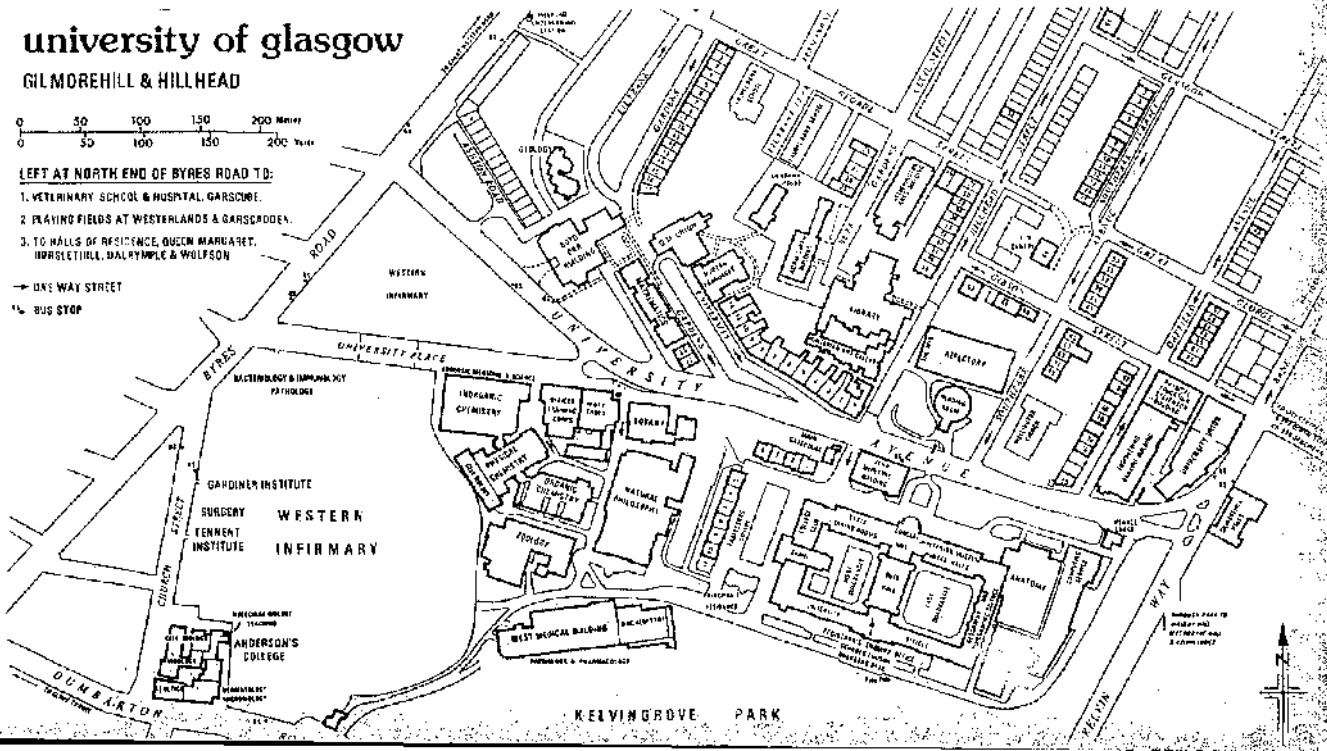
Our body is a machine which needs energy, in the form of food, to work; the amount of energy (calories) required, depends on how much energy the body expends for its essential functions (Basal Metabolic Rate = B M R) and for physical activities.

B M R is the basis of all calculations of energy expenditure, and one of the main factors that can affect its value is body composition (amount of fat and muscle).

We are investigating the effect of lean muscular body composition on B M R , in women and men.

If you are slim with a high proportion of muscle and are interested in knowing about your energy needs and body composition YOU CAN HELP US ! The study takes place in the morning, from about 9 to 12. All measurements are naturally safe and entirely painless.

Please fill in the slip attached and send it to TERESA ESPINOSA Institute of Physiology, room 503; if you have any enquiries, please ring me on 041 - 339 88 55 ext. 6614 or 4765 . Thank you!



INSTITUTE OF PHYSIOLOGY
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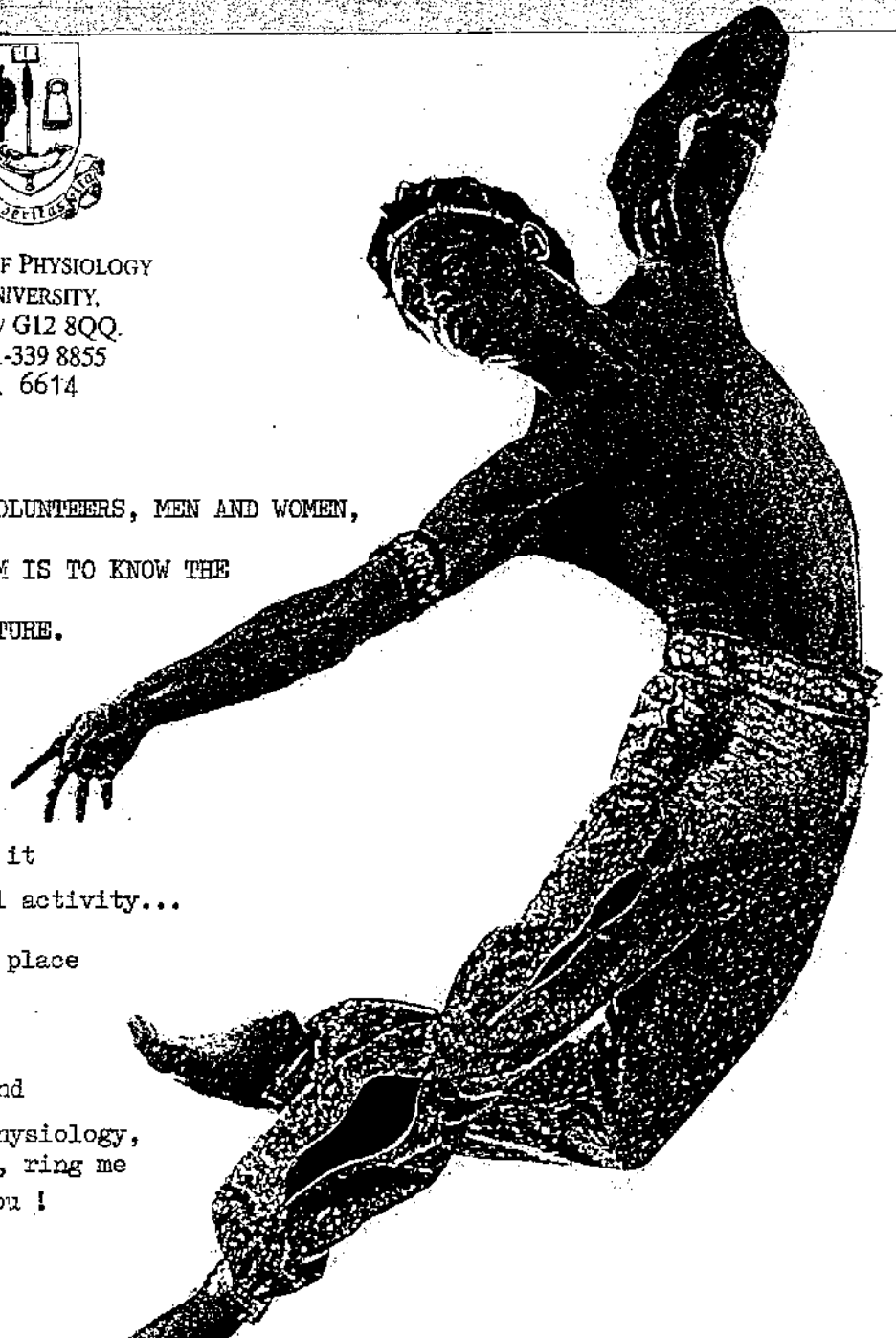
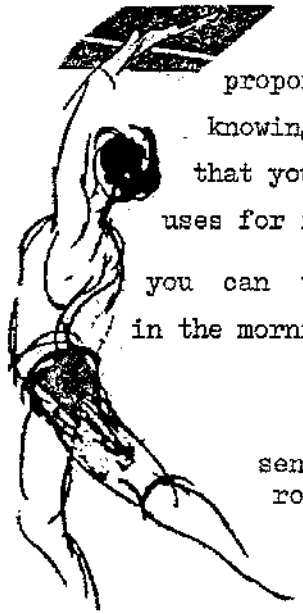
EXT. 6614



WE ARE LOOKING FOR LEAN MUSCULAR VOLUNTEERS, MEN AND WOMEN, TO TAKE PART IN A RESEARCH PROJECT WHICH AIM IS TO KNOW THE EFFECT OF BODY COMPOSITION ON ENERGY EXPENDITURE.



If you are lean with a high proportion of muscle and are interested in knowing about the amount of fat and muscle that your body has, and the number of calories it uses for its essential functions and for physical activity... you can take part in this study ! It takes place in the morning and lasts three hours.

Please fill in the slip attached and send it to: Teresa Espinosa. Institute of Physiology, room 503; also, if you have any enquiries, ring me on the above telephone . Thank you !

PROFESSOR J.V.G.A. DURNIN
MA, MB ChB, DSc, FRCP, FIBiol, FRSE
DIRECT LINE: 041-330 4612
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EXT.

July, 1989

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Dear

We are carrying out a study to determine the most appropriate techniques to assess muscularity and fatness and its relationship to energy expenditure in lean muscular people.

The reason for this study is that none of the standard techniques for these measurements are entirely valid for this type of individual. Since, in populations in many developing countries where undernutrition may be common, it is often important to measure the small reserves of fat contained in their body, the development of more accurate measurements has much importance. It may seem strange to compare a fit young athlete in this country with people in countries like India, but both types, from the aspect of relative muscularity and fatness, have features which have some similarities.

The measurements we need to make are:

1) basal metabolic rate
2) energy expenditure while walking on a treadmill
3) body density (by weighing the individual in a tank of water)
4) skinfold thicknesses
5) total body water
6) total body potassium

The entire study takes 3 hours on any morning here at the Institute of Physiology and another one hour to measure potassium which is carried out at the National Engineering Laboratory in East Kilbride.

This study will give information to all participating subjects about the amount of fat and muscle in their body and their energy metabolism.

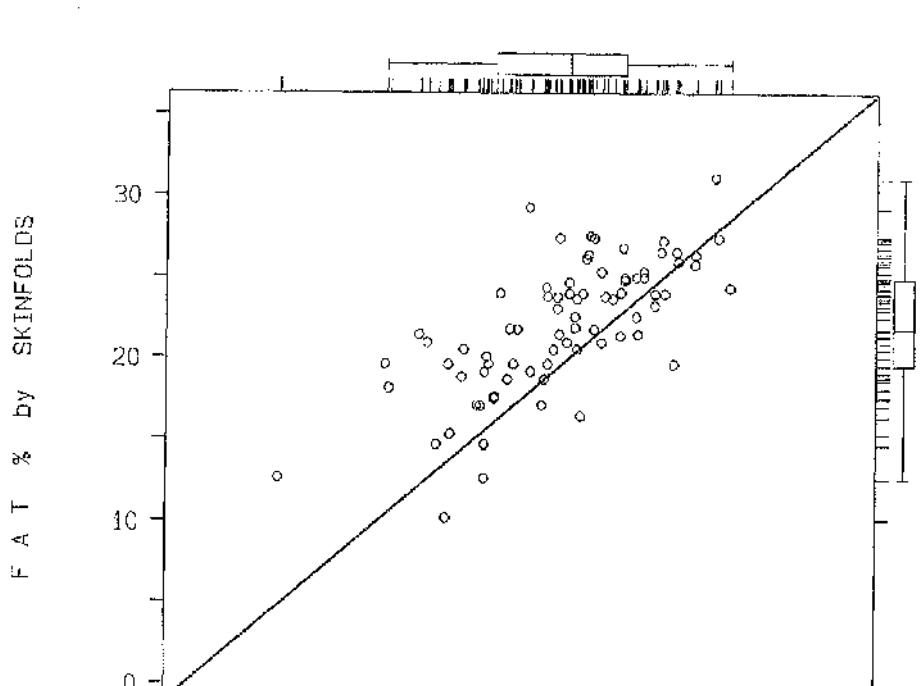
It would be most kind of you to put us in touch with any of your athletes who might be suitable and we can, of course, explain anything to them in more detail. Thank you very much for any help you could give us.

Looking forward to hearing from you.

Yours sincerely,

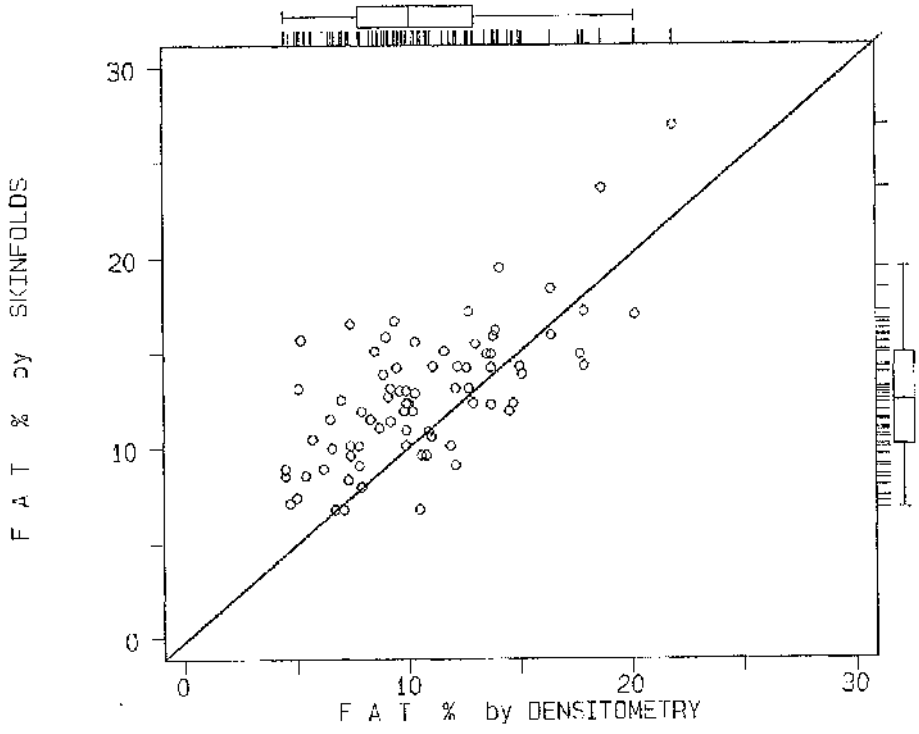
J.V.G.A. Durnin Dr. Teresa Espinosa Zepeda

APPENDIX 2



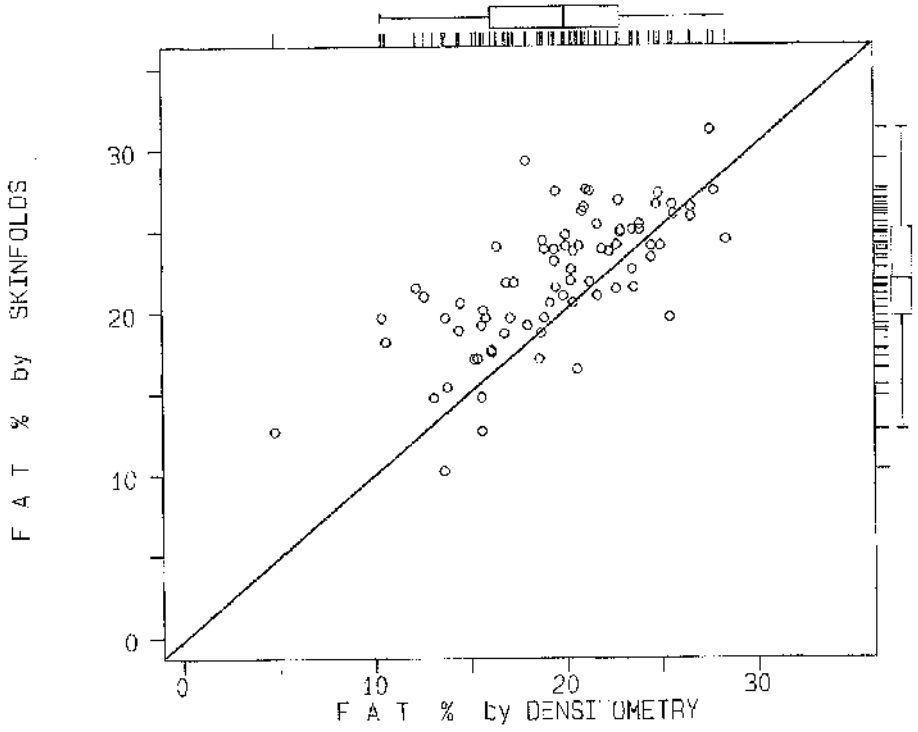
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=78)	19.6 ± 4.55	4.8	16.1	20.0	22.9	28.4
Skinfolds (n=78)	22.0 ± 4.05	10.2	19.6	22.2	25.0	31.1

Figure 1. Comparison of fat% between densitometry and skinfolds for females (n=78; $R_1æ$ = 0.05).



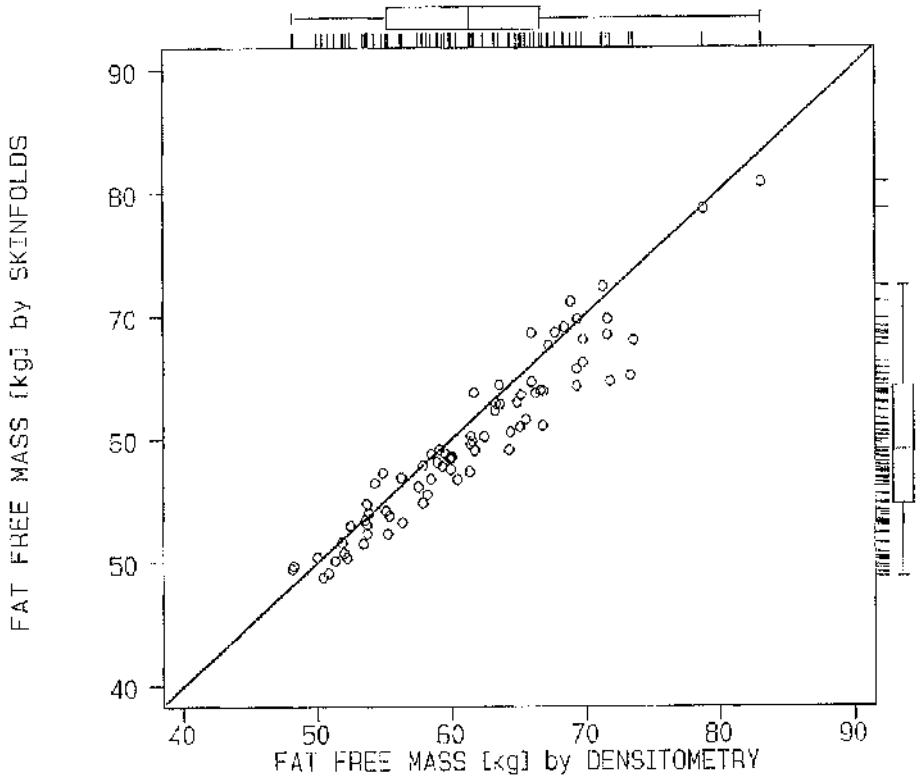
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=78)	10.7 ± 3.84	4.5	7.8	10.1	13.0	21.9
Skinfolds (n=78)	12.7 ± 3.61	6.7	10.1	12.4	14.9	27.0

Figure 2. Comparison of fat% between densitometry and skinfolds for males (n=78; $R_1æ$ = 0.05).



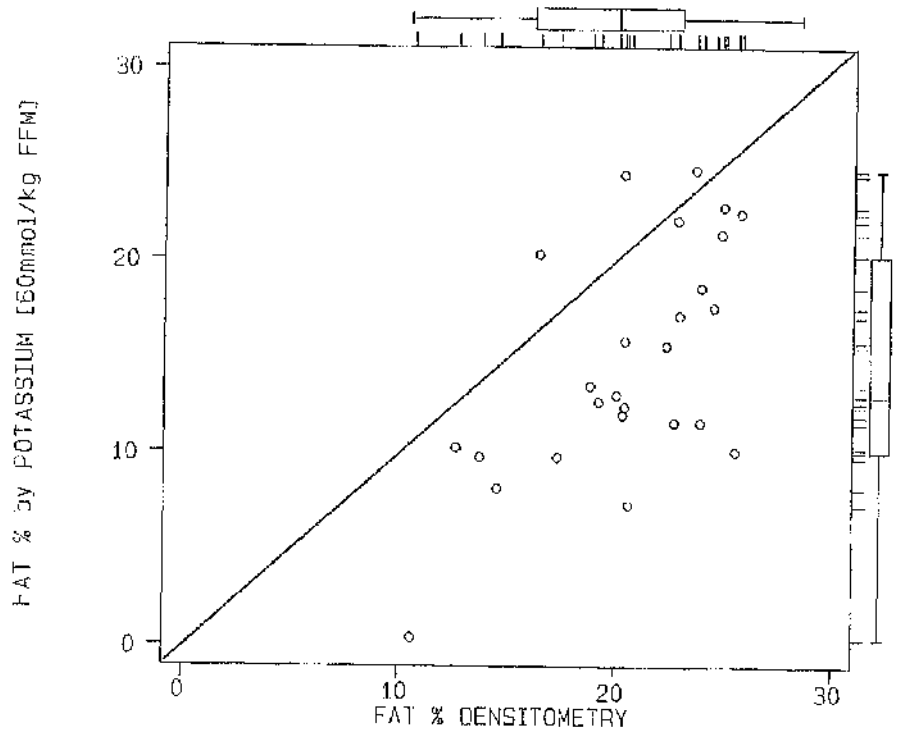
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=78)	44.4 ± 5.04	31.7	41.4	44.7	47.6	54.1
Skinfolds (n=78)	43.1 ± 4.65	30.2	40.3	42.4	47.2	52.0

Figure 3. Comparison of fat free mass [kg] between densitometry and skinfolds for females (n=78; $R_1æ$ = 0.23).



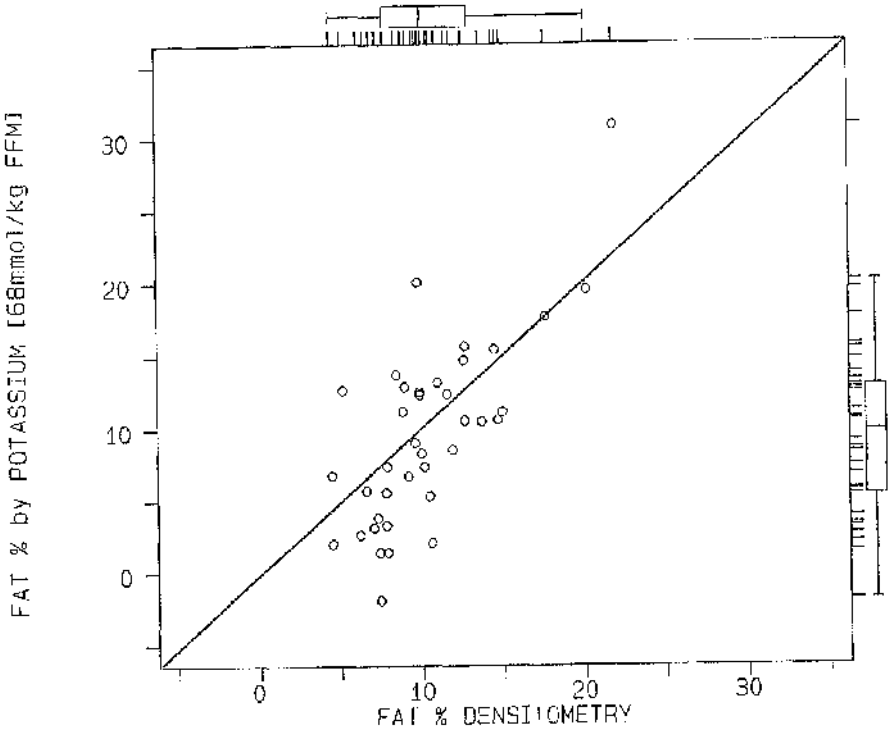
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=78)	61.4 ± 7.28	48.1	55.3	61.3	66.6	83.1
Skinfolds (n=78)	59.9 ± 6.83	48.8	54.8	59.1	64.3	80.8

Figure 4. Comparison of fat free mass [kg] between densitometry and skinfolds for males (n=78; $R_1æ$ = 0.37).



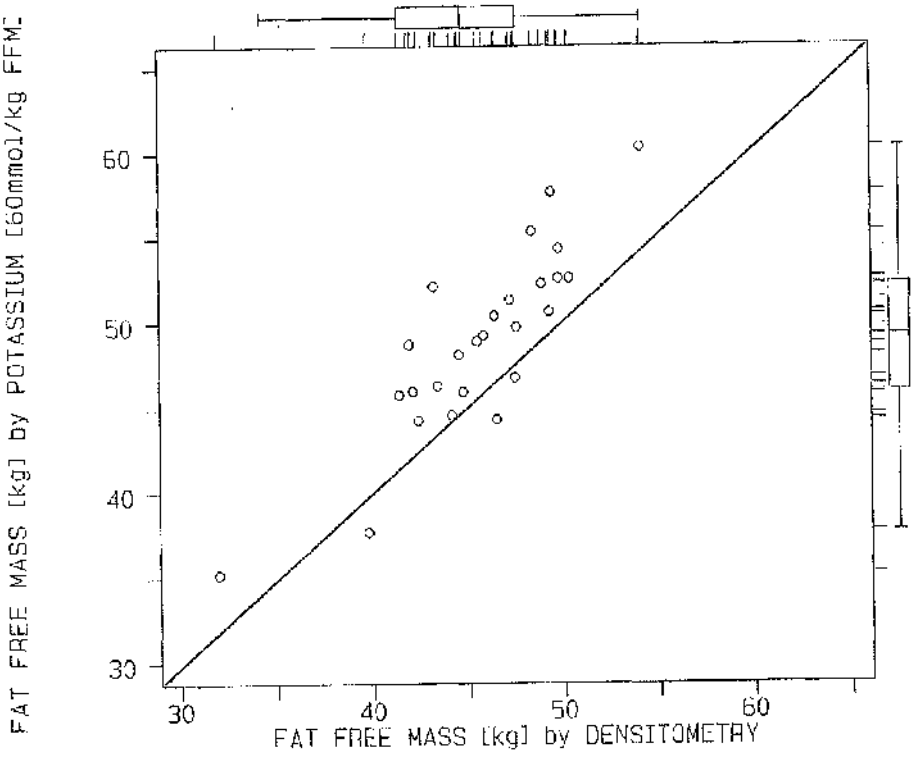
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=26)	20.5 ± 4.16	10.6	18.8	20.5	23.9	25.7
Potassium (60mmol n=26	14.8 ± 6.05	0.4	10.3	13.3	20.3	24.7

Figure 5. Comparison of fat% between densitometry and potassium for females (n=26; $R_1æ$ = 0.07).



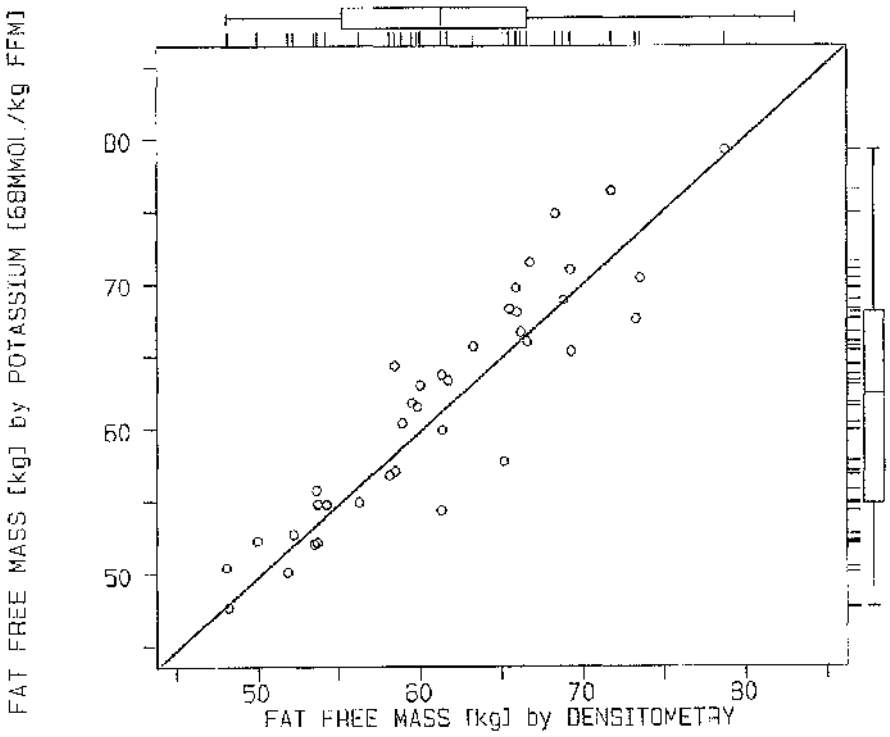
METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=38)	10.4 ± 3.94	4.5	7.8	9.9	12.6	21.9
Potassium (n=38)	9.6 ± 6.42	-2.0	5.3	9.7	12.9	30.8

Figure 6. Comparison of fat% between densitometry and potassium for males (n=38; R_1 æ = 0.48).



METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=26)	45.6 ± 4.35	32.0	43.2	46.1	48.9	54.1
Potassium (n=26)	48.9 ± 5.38	35.2	45.9	49.1	52.3	60.3

Figure 7. Comparison of fat free mass [kg] between densitometry and potassium for females (n=26; $R_1æ$ = 0.12).



METHOD	MEAN ± S.D.	min	p 25%	p 50%	p 75%	max
Densitometry (n=38)	61.4 ± 7.52	48.1	54.3	61.3	66.6	78.7
Potassium (n=38)	61.9 ± 8.02	47.7	52.1	62.4	68.0	79.2

Figure 8. Comparison of fat free mass [kg] between densitometry and potassium for males (n=38; R_1æ = 0.86).

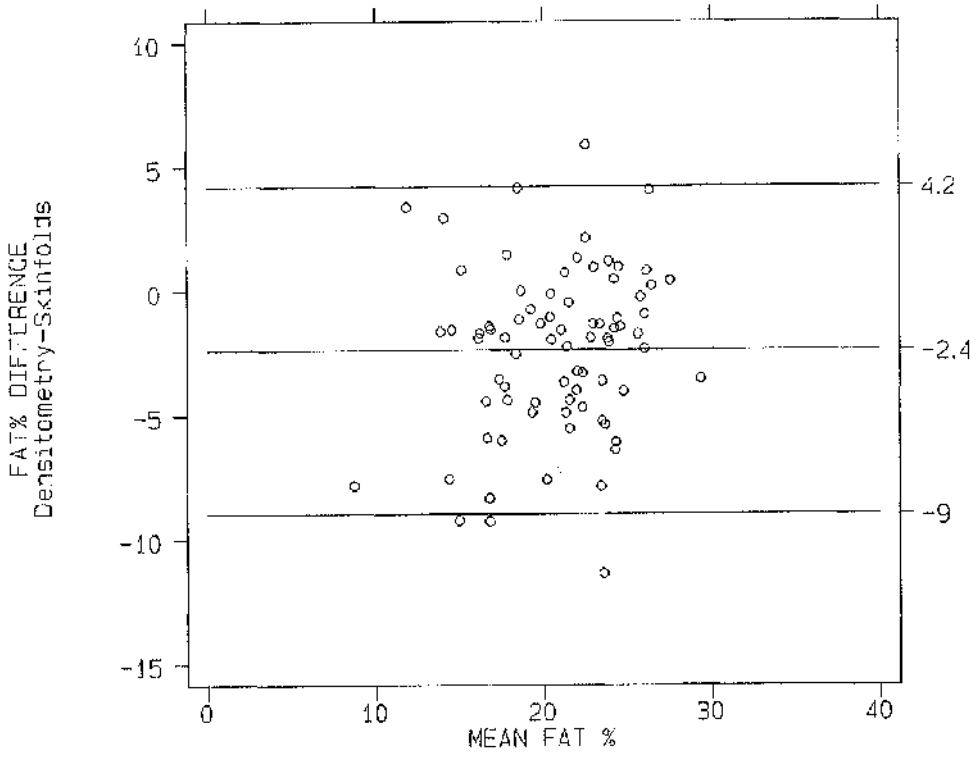


Figure 9. Difference against mean for fat% by the methods of densitometry and skinfolds for females (n = 78).
The three lines represent the mean difference ± 2 S.D.

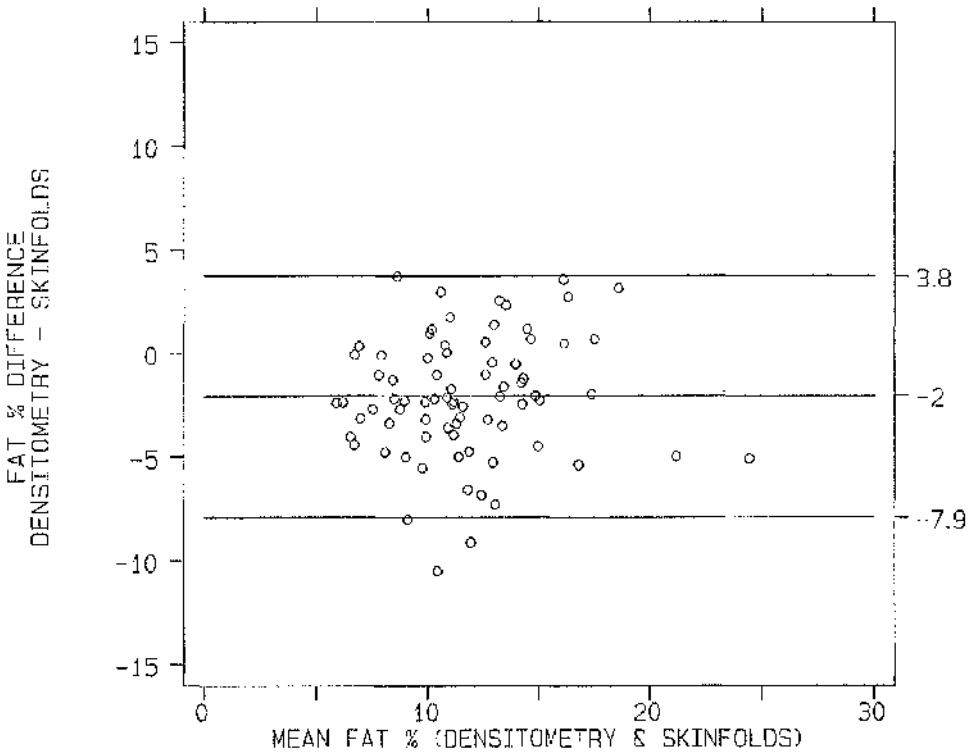


Figure 10. Difference against mean for fat% by the methods of densitometry and skinfolds for males (n = 78).

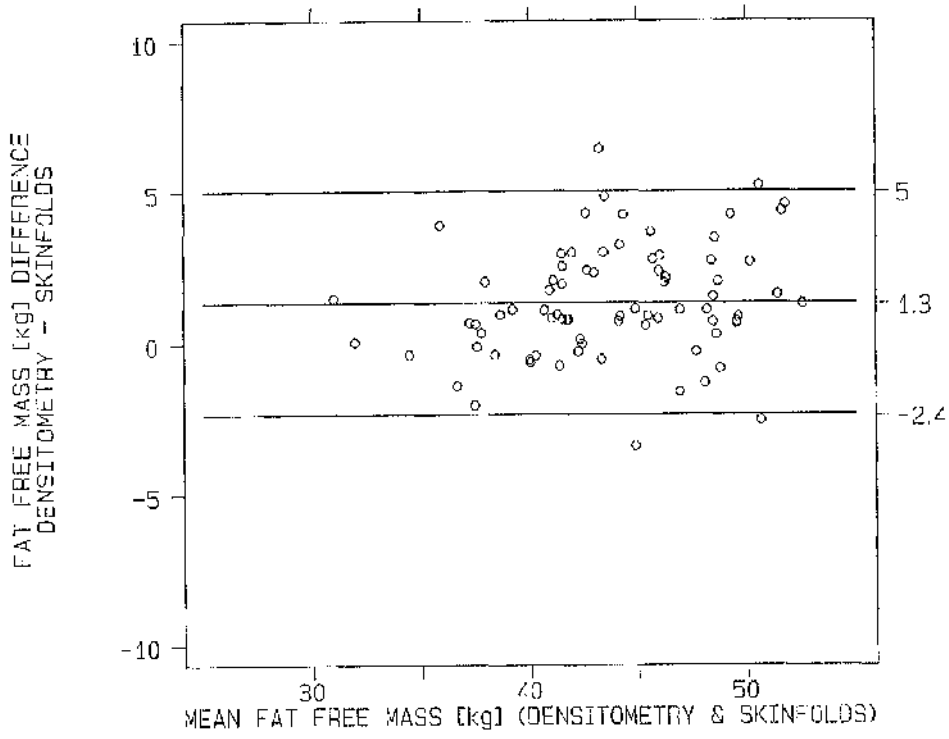


Figure 11. Difference against mean for fat free mass [kg] by the methods of densitometry and skinfolds for females (n= 78).

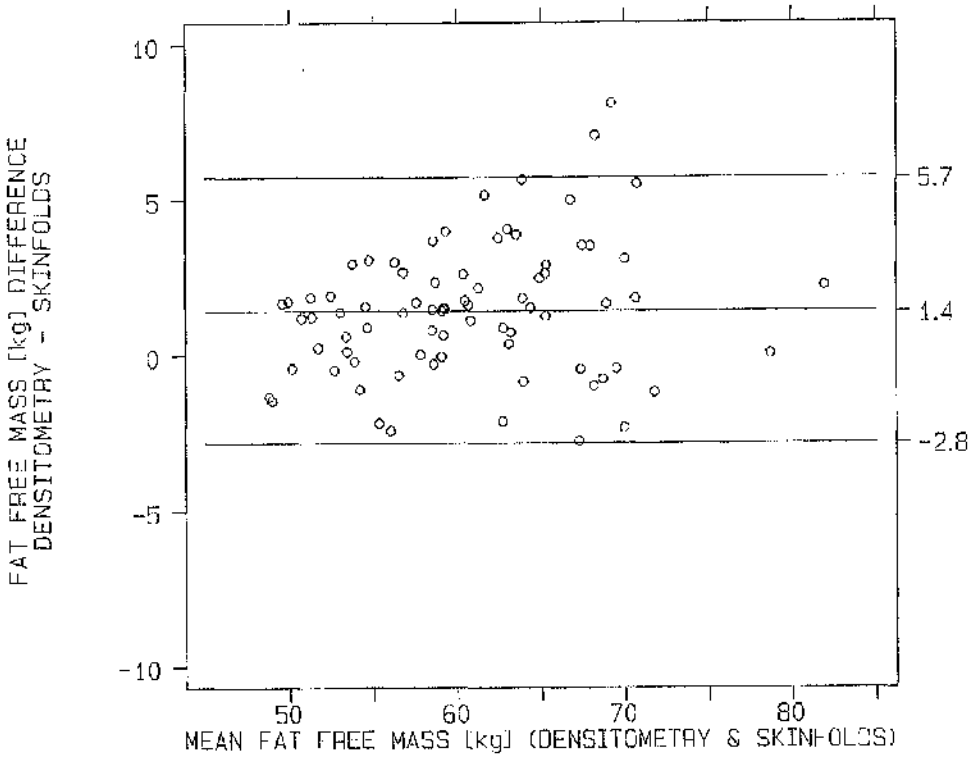


Figure 12. Difference against mean for fat free mass [kg] by the methods of densitometry and skinfolds for males (n = 78).

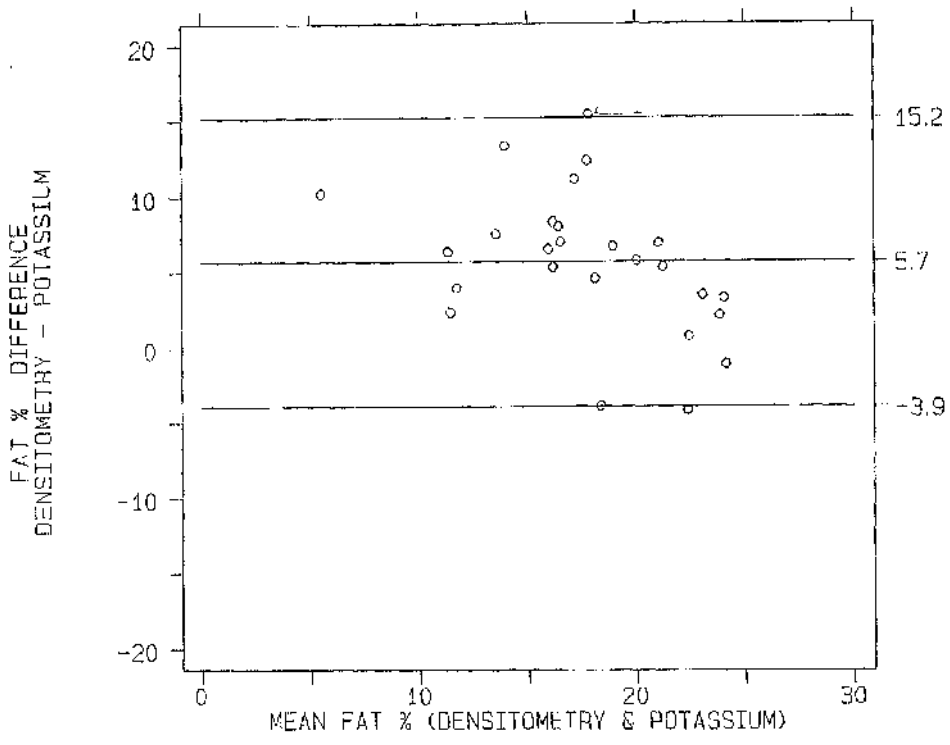


Figure 13. Difference against mean for fat% by the methods of densitometry and potassium for females (n = 26).

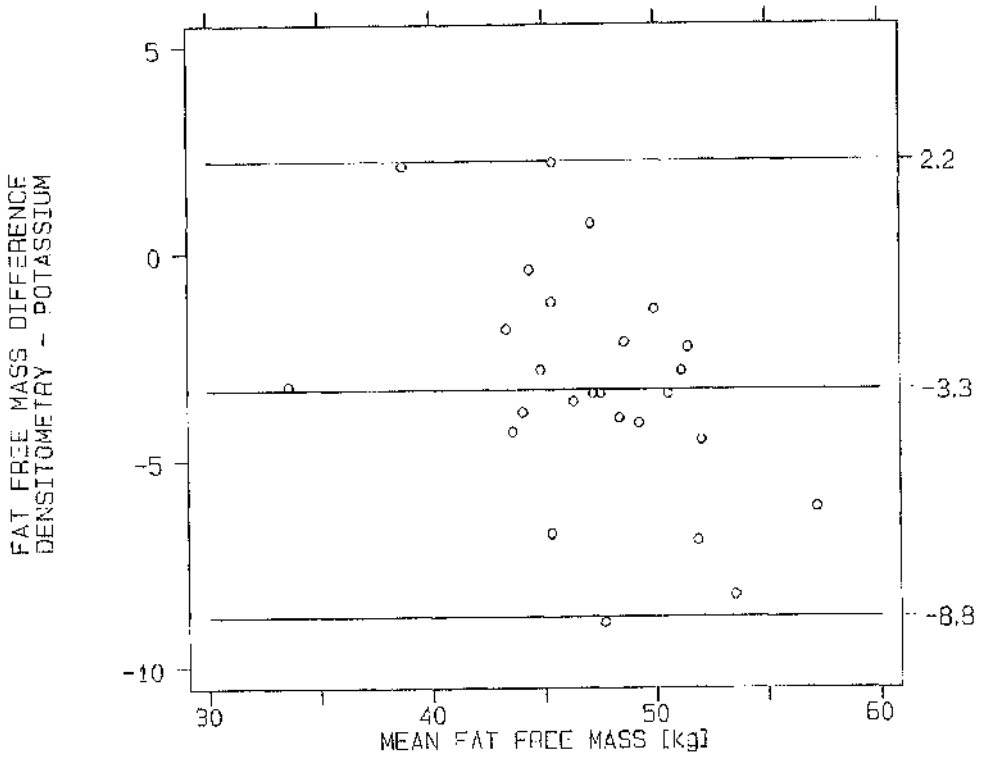


Figure 14. Difference against mean for fat free mass [kg] by the methods of densitometry and potassium for females (n = 26).

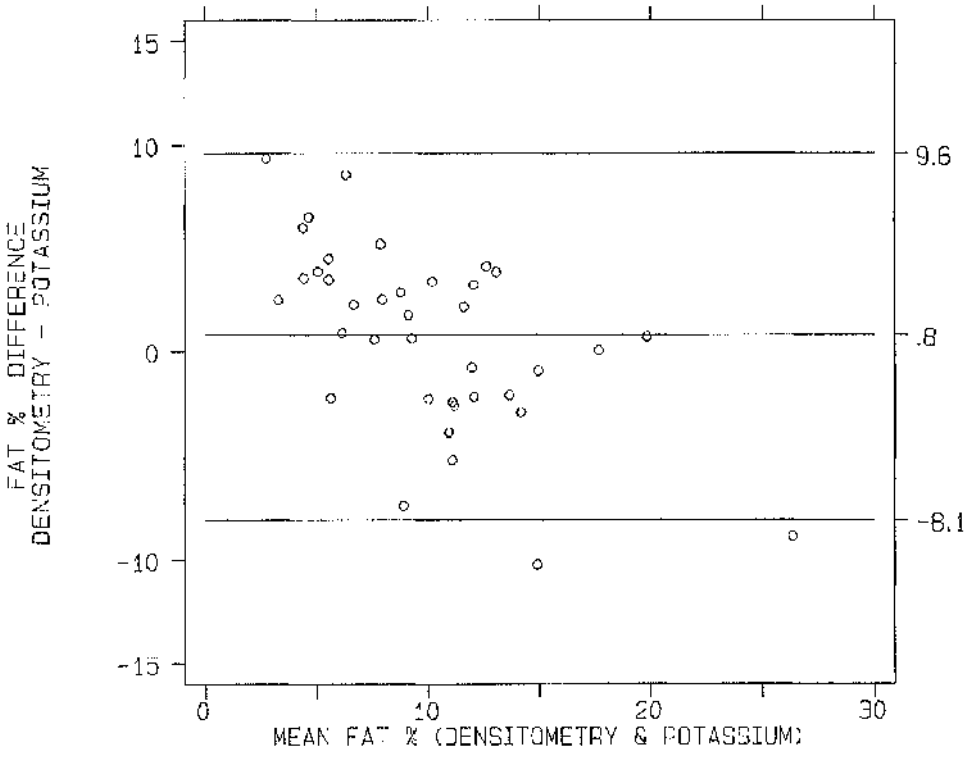


Figure 15. Difference against mean for fat% by the densitometry and the potassium methods for males (n = 38).

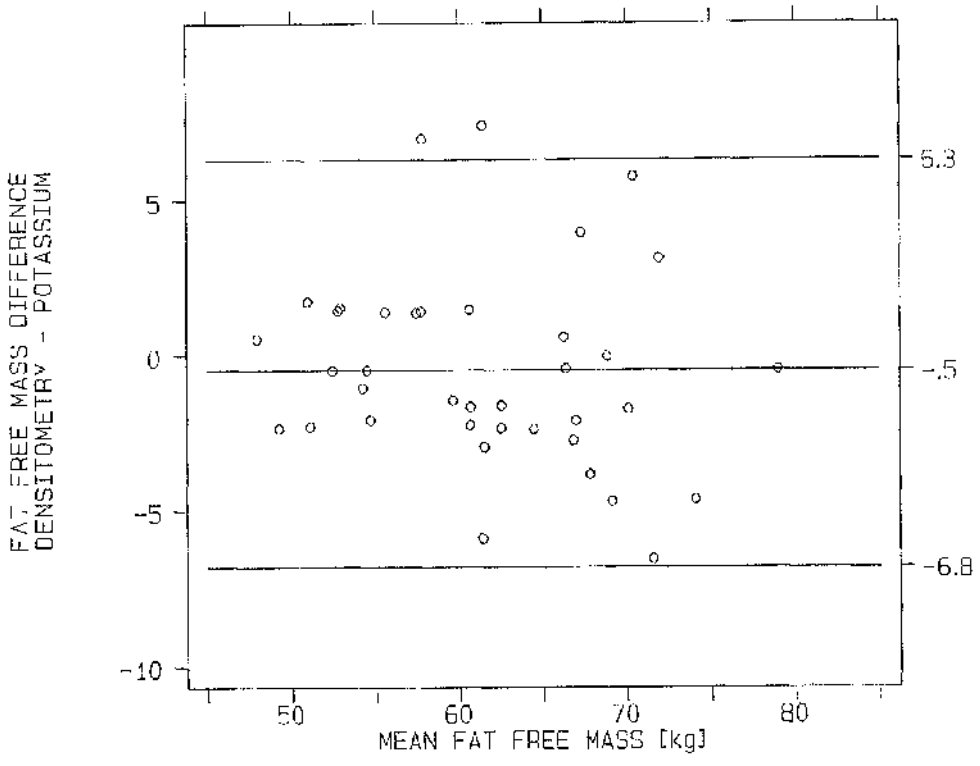


Figure 16. Difference against mean for fat free mass [kg] by the densitometry and potassium methods for males (n = 38).

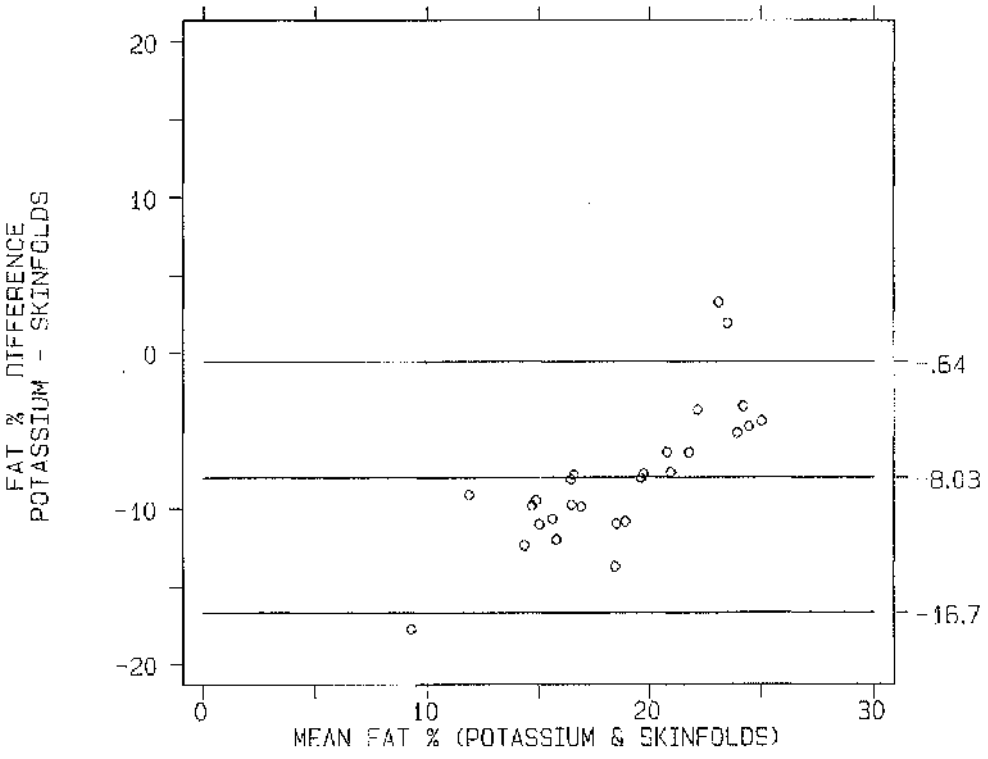


Figure 17. Difference against mean for fat% by the methods of potassium and skinfolds for females (n = 26).

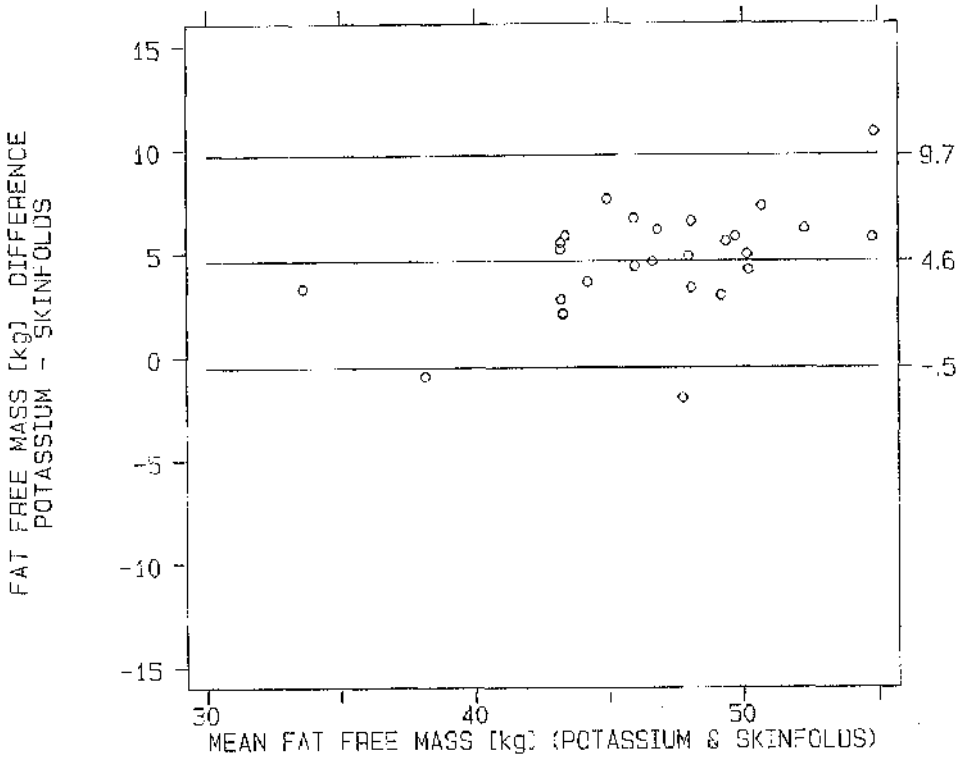


Figure 18. Difference against mean for fat free mass [kg] by the methods of potassium and skinfolds for females (n= 26).

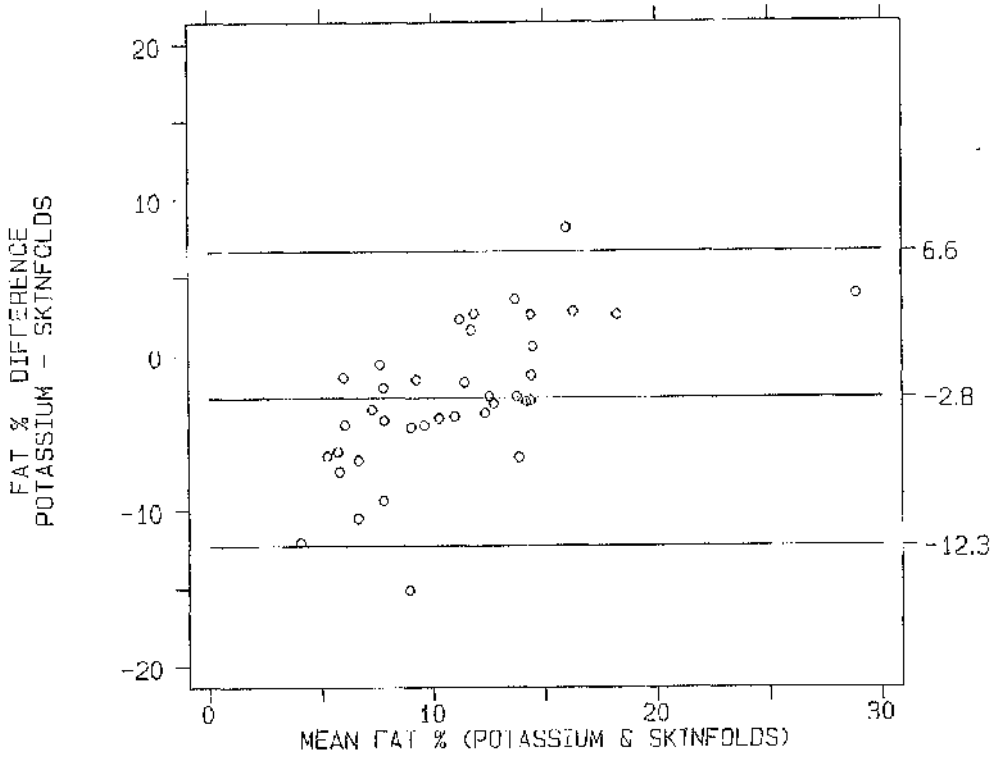


Figure 19. Difference against mean for fat% by the potassium and the skinfolds methods for males (n = 38).

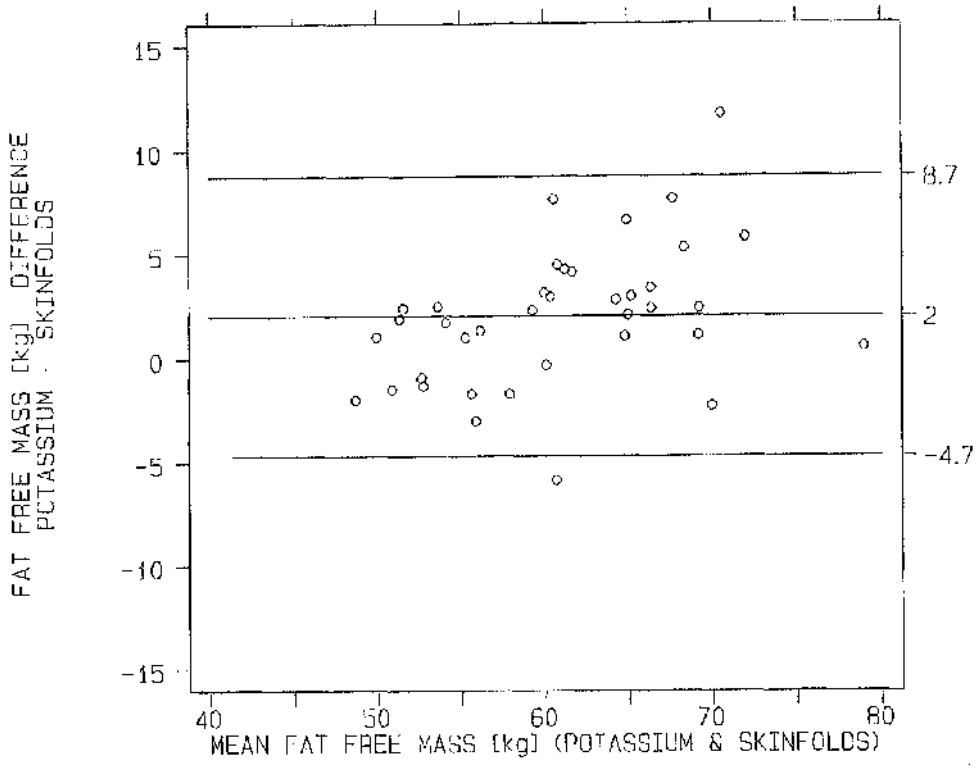


Figure 20. Difference against mean for fat free mass [kg] by the methods of potassium and skinfolds for males (n = 38).

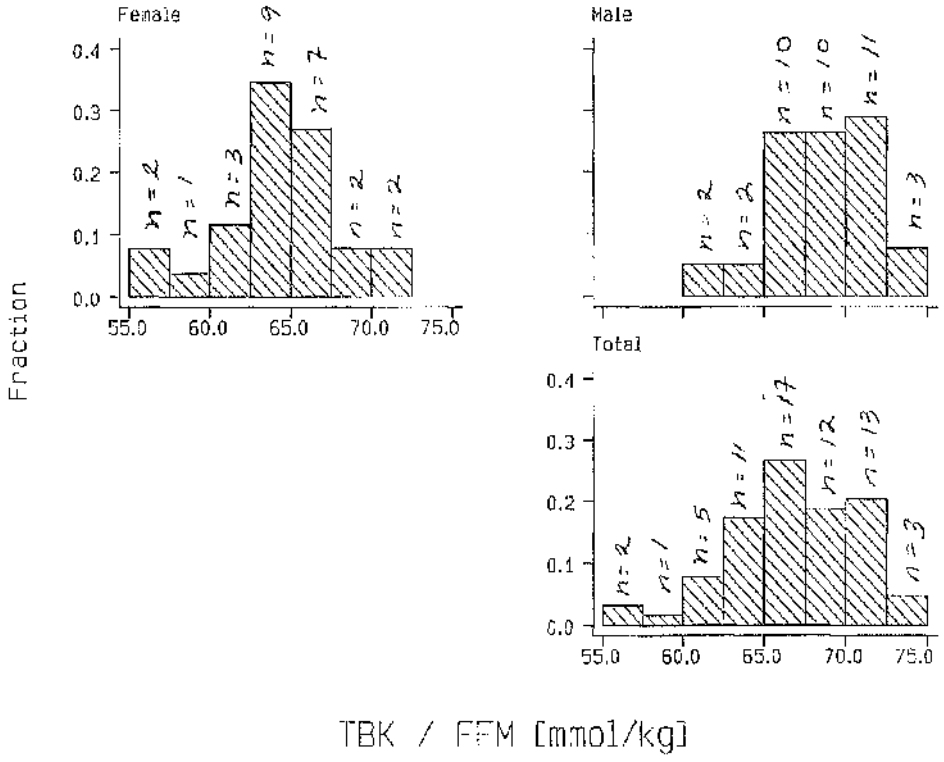


Figure 21. Histogram of the ratios of total body potassium expressed per kilogram of fat free mass. Females (n=26) & males (n=38).

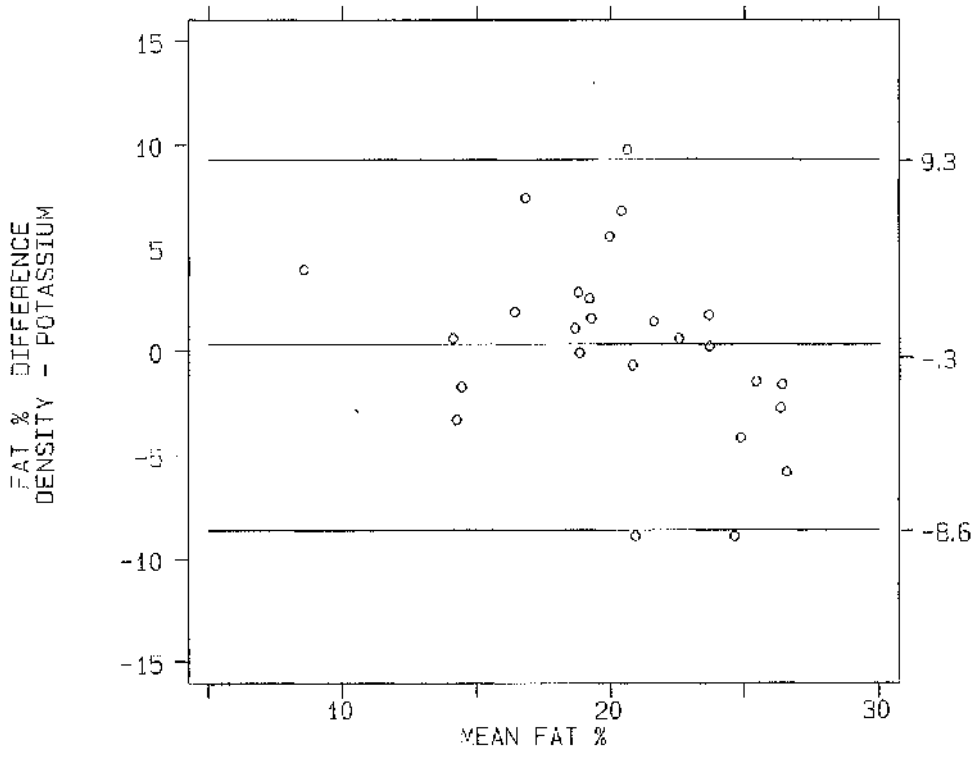


Figure 22. Difference against mean for fat% by the methods of densitometry and potassium (64 mmol/kg FFM) for females (n = 26).

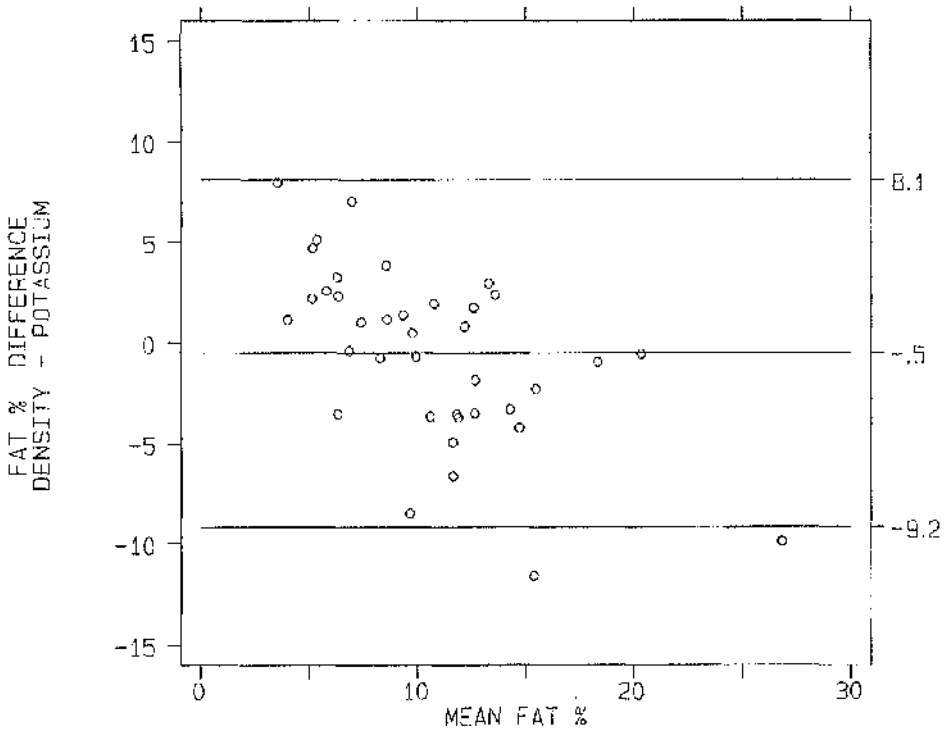


Figure 23. Difference against mean for fat% by the methods of densitometry and potassium (69 mmol/kg \FFM) for males (n = 38).

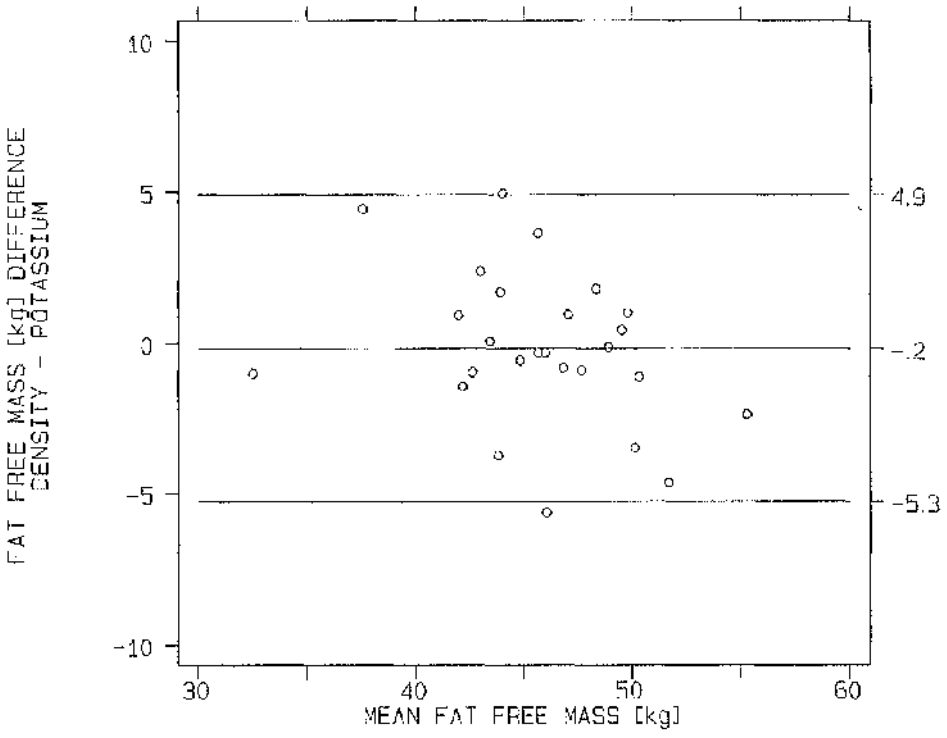


Figure 24. Difference against mean for fat free mass [kg] by the methods of densitometry and potassium (64 mmol/kg) for females (n=26)

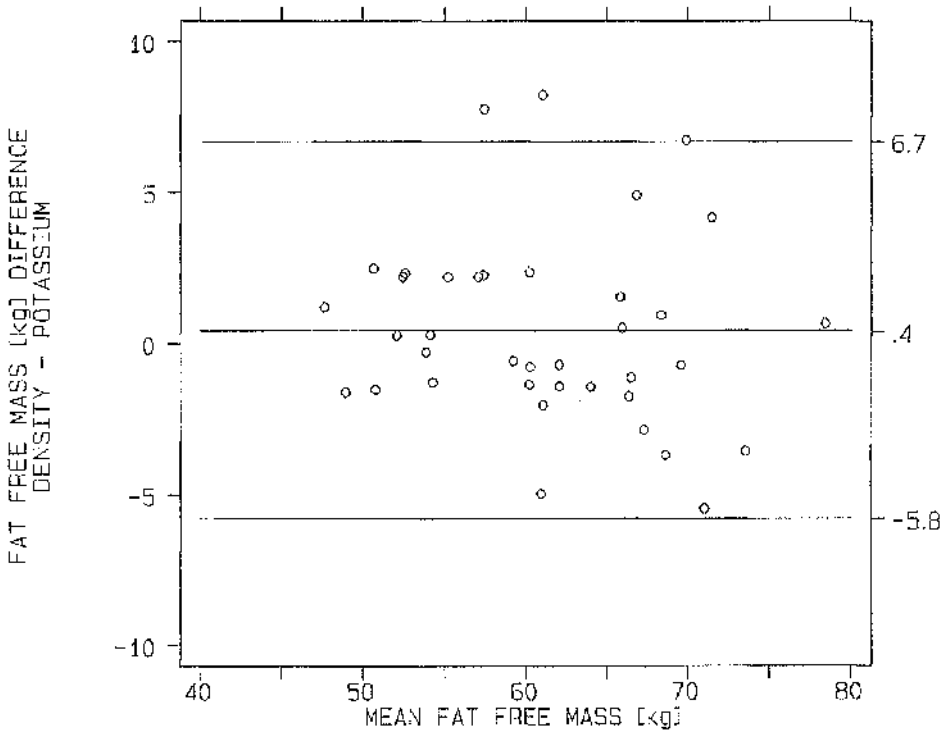


Figure 25. Difference against mean for fat free mass [kg] by the methods of densitometry and potassium (69mmol/kg) for males (n=38).

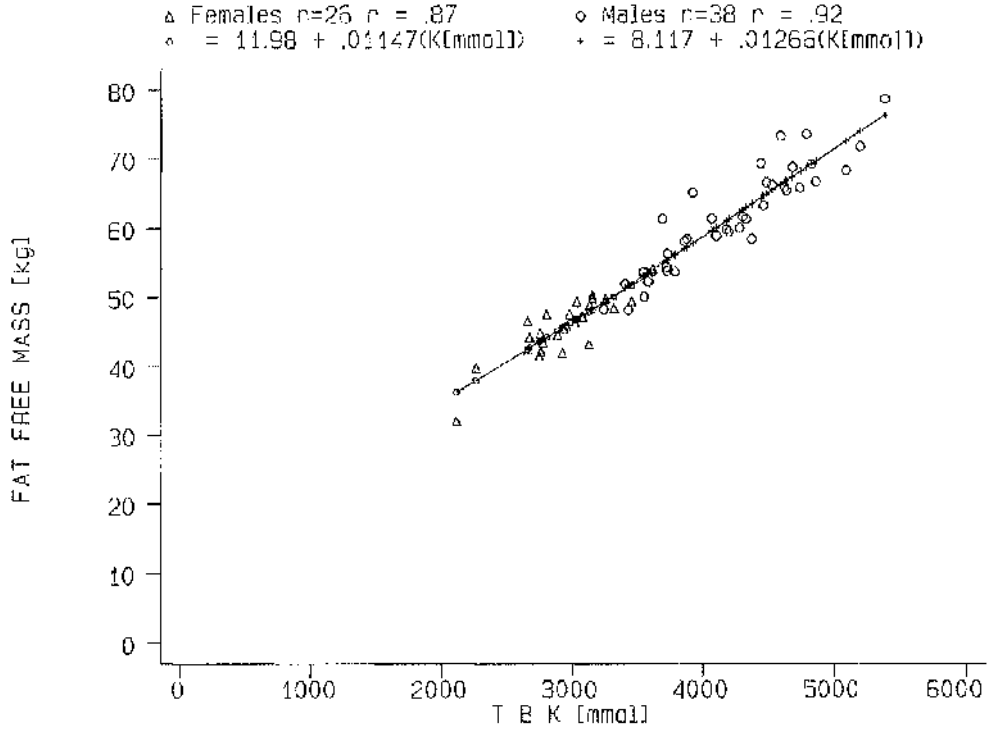


Figure 26. Scatter and linear regression of fat free mass on total body potassium for females° (n=26) & males⁺ (n=38).

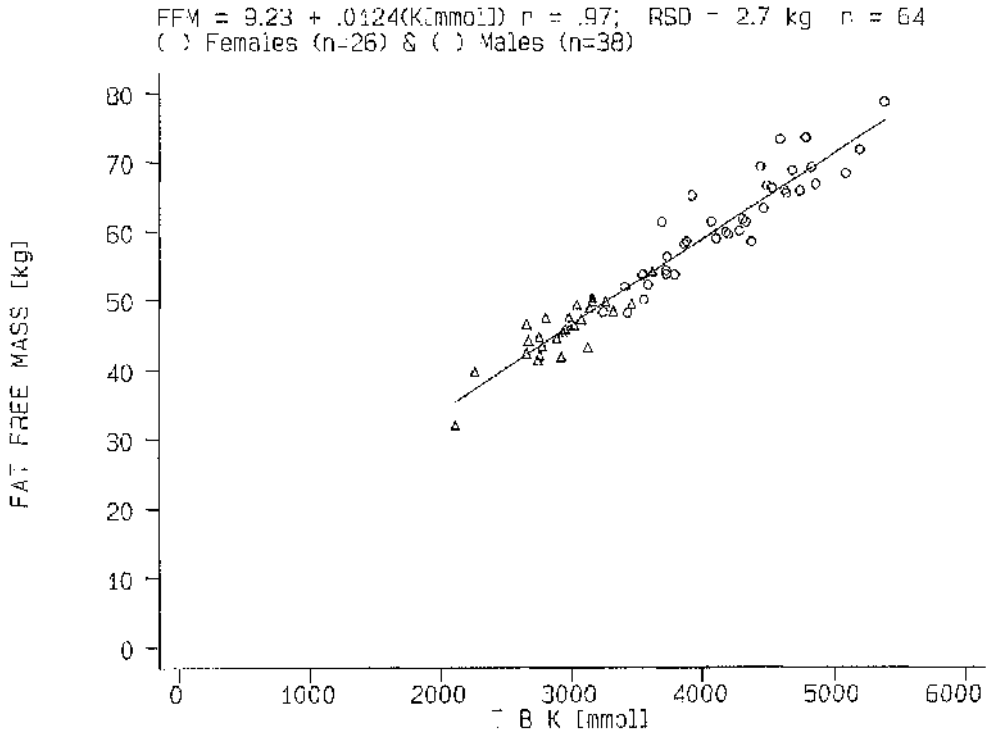


Figure 27. Scatter & common linear regression of fat free mass on total body potassium for both sexes.

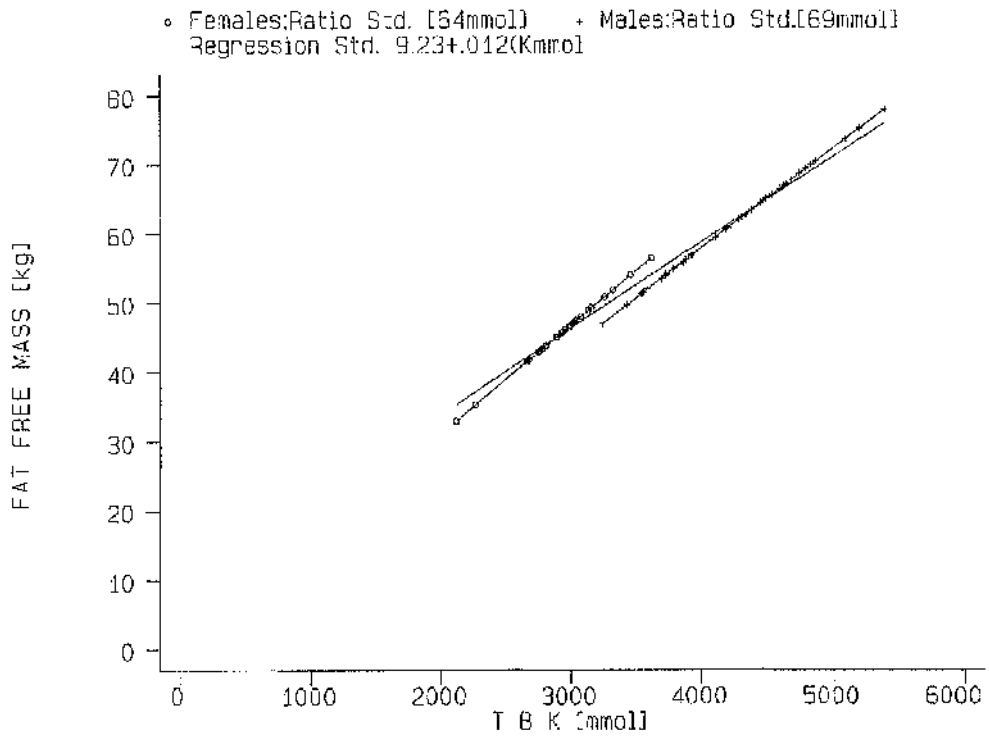


Figure 28. Relationship between FFM [kg] and total body potassium [mmol].
Linear regression line (___) and hypothetical ratio standard line (...) for both sexes (n = 64).

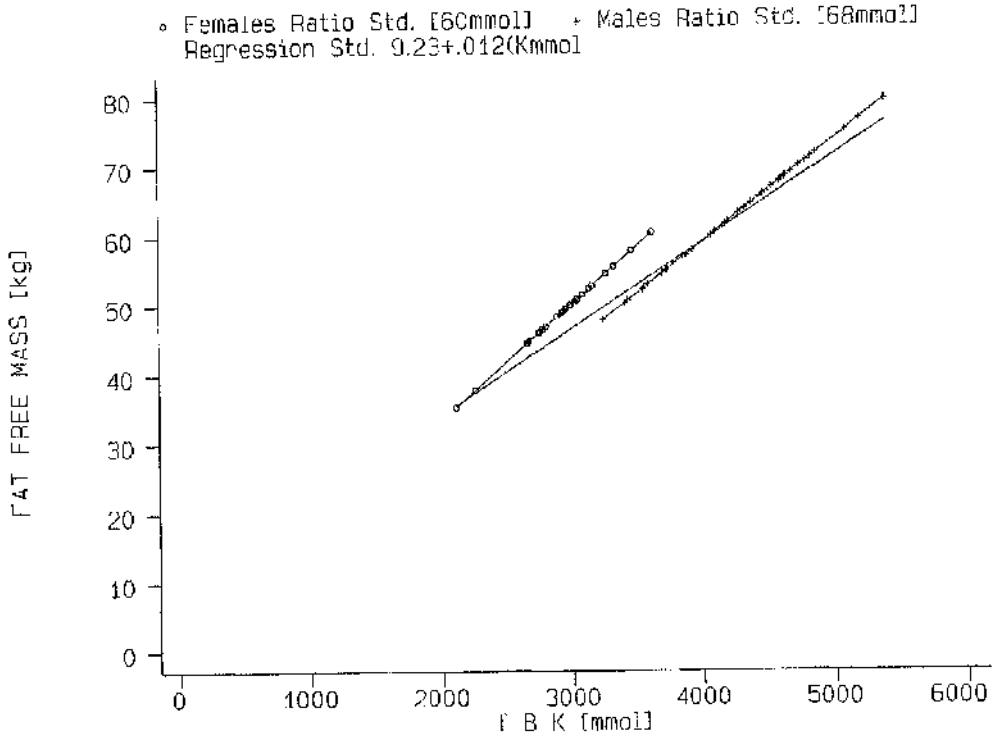


Figure 29. Relationship between fat free mass and total body potassium.
Linear regression line for both sexes (n=64) and hypothetical ratio standard line for females [60mmol/kg FFM] (n = 26) and for males [68mmol/kg FFM] (n=38).

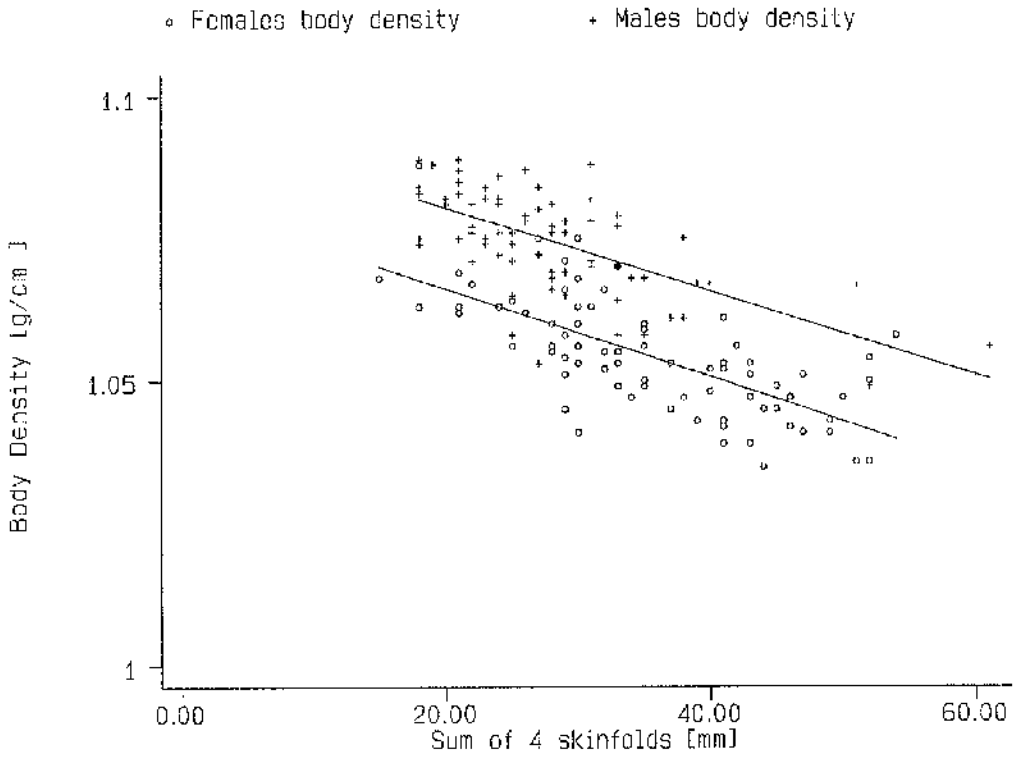


Figure 30. Regressions for the log of sum of 4 skinfold thickness and whole body density for females and for males.

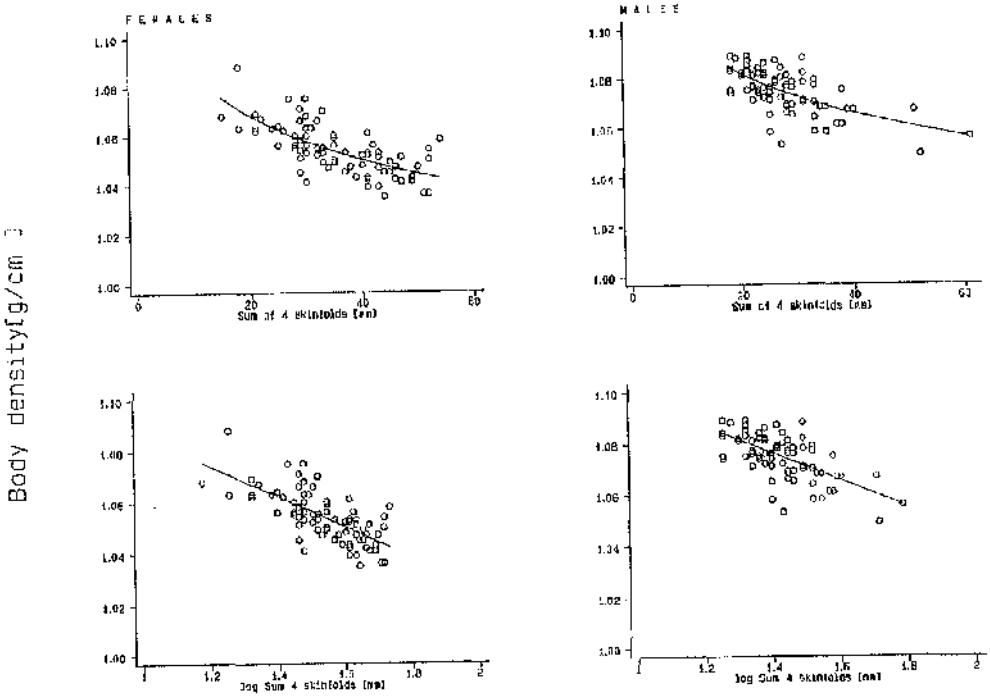


Figure 31. Individual values for body density and sum of 4 skinfolds with best-fit regression line derived from log values of skinfolds.

REFERENCES

Baldwin, R.L., & Smith, N.E. (1971) Application of a simulation modeling technique in analysis of dynamic aspects of animal energetics. *Federation Proceedings Federal American Society of Experimental Biology*, **30**, 1459.

Baker, P.T. & Angel, J.L. (1965) Old age changes in bone density: sex and race factors in the United States. *Human Biology*, **37**, 104-121.

Barengolts, E.I., Curry, D.J., Bapna, M.S. & Kukreja S.C. (1993) Effects of endurance exercise on bone mass and mechanical properties in intact and ovariectomized rats. *Journal of Bone and Mineral Research*, **8**, 937-942.

Barrows, K. & Snook, J.T. (1987) Effect of high-protein, very-low-calorie diet on resting metabolism, tyroid hormones, and energy expenditure of obese middle aged women. *American Journal of Clinical Nutrition*, **45**, 391-398.

Bedell, G.N. Marshall R., Debois A.B. & Harris, J.H. (1956) Measurement of the volume of gas in the gastrointestinal tract. Values in normal subjects and ambulatory patients. *Journal of Clinical Investigation*, **35**, 366-345.

Behnke, A.R., Feen, B.G. & Welham W.C. (1942) The specific gravity of healthy men. *Journal of the American Medical Asociation*, **118**, 495-498.

Benedict, F.G. (1915) Factors affecting basal metabolism. *Journal of Biological Chemistry*, **20**, 263-299.

Bernstein , R.S., Thornton, J.C., Yang, M.U., Wang, J., Redmon, A.M., Pierson, R.N., Pi-Sunyer, F.X. & Van Itallie, T.B. (1983) Prediction of resting metabolic rate in obese patients. *American Journal of Clinical Nutrition*, **37**, 595-602.

Bessard, T., Schultz, Y. & Jequier, E. (1983) Energy expenditure and postprandial thermogenesis in obese women before and after weight loss. *American Journal of Clinical Nutrition*, **38**, 680-693.

Bisdee,, J.T., James, W.P.T., & Shaw, M.A. (1989) Changes in energy expenditure during menstrual cycle. *British Journal of Nutrition*, **61**, 187-199.

Bland M.J. & Altman D.G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, 307-310.

Boddy, K., Elliott, A., Robertson, I., Mahaffy, M., & Holloway, I. (1975) A high sensitivity dual-detector shadow-shield whole body counter with an 'invariant' response for total body "in vivo" neutron activation analysis. *Physical Medical Biology*, **20**:2, 296-304.

Boddy, K., King, P.C., Tothill, P., & Stron, J.A. (1971) Measurement of total body potassium with a shadow shield whole body counter: calibration and errors. *Phys. Med. Biol.*, **16**:2, 275-282.

Boothby, W.M. & Sandiford, I. (1929) Normal values for standard metabolism. *American Journal of Physiology*, 90, 290-291.

Boothby, W.M., Berkson, J., & Dunn, H.L. (1936) Studies of the energy metabolism of normal individuals: a standard for basal metabolism, with a nomogram for clinical applications. *American Journey of Physiology*, **116**, 468-484.

Brody, S. (1945) Bioenergetics and Growth, Hafner Press, New York. In Crist, K.A., Baldwin, R.L. and Stern, J.S. Energetics and the demands for maintenance. *Human Nutrition. A comprehensive treatise. 3A Nutrition and the adult: macronutrients*, ed. Alfin-Slater R.B. & Kritchevsky, D. (1980) Ch. 6, pp 159-182. Plenum Press, New York.

Brown, W. & Jones PRM. (1977) The distribution of fat in relation to habitual activity. *Annals of Human Biology*, **4**:6, 537-550.

Brozek, J. & Keys, A. (1951) The evaluation of leanness fatness in man: norms and interrelationships. *British Journal of Nutrition*, 5, 194-206.

Burkinshaw, L. & Cotes, J.E. (1973) Body potassium and fat-free mass. *Clinical Science*, **44**, 621-622.

Buskirk, E.R., Iampietro,P.F., & Welch, B.E., (1957) Variations in resting metabolism with changes in food, exercise and climate. *Metabolism*, **6**,144.

Cavanaugh, D.J. & Cann, C.E. (1988). Brisk walking does not stop bone loss in post-menopausal women. *Journal of Bone & Mineral Research*, **9**, 201-204.

Cheek, D.B. (1968). Body potassium and lean body mass. In *Human Growth*, ed. Cheek, D.B. Philadelphia: Lee & Febiger.

Clarys, J.P., Martin, A.D. & Drinkwater D.T. (1984) Gross tissue weights in the human body by cadaver dissection. *Human Biology*, 56, 459-473.

Clarys , J.P. & Martin, A.D. (1985) The concept of the adipose tissue-free mass. In *Human Body Composition and Fat distribution*, ed Norgan, N.G. Euro Nut report 8: 49-63. Stichting Voeding Nederland, Wageningen.

Clarys, J.P., Martin, A.D., Drinkwater D.T. & Marfell-Jones, M.J. (1987) The skinfold: myth and reality. *Journal of Sports Science*, 5, 3-33.

Colton, T. (1974) Regression and Correlation. In *Statistics in Medicine*. Ch. 6. Little, Brown and Company (Inc.) Boston. pp 207-212.
Consolazio, C.F., R. Shapiro, J.E. Masterson, & P.S.L. McKinzie. (1961) Energy requirements of men in extreme heat. *Journal of Nutrition*, 73:126-134.

Consolazio, C.F., R.E. Johnson & L.J. Pecora (1963) *Physiological measurements of metabolic functions in man*. New York: McGraw Hill.

Coward, W.A. In Prentice, AM (ed, 1990) The doubly-labelled water method: technical recommendations for use in humans. Report of an IDECG ~~expert working group. in press~~. Technical Document IAEA

Crist, K.A., Baldwin, R.L. & Stern, J.S. (1980) Energetics and the demands for maintenance. In *Human Nutrition. A comprehensive treatise. 3A Nutrition and the adult: macronutrients*, ed. Alfin-Slater R.B. & Kritchevsky, D. Ch. 6, pp 159-182. Plenum Press, New York.

Cunningham, J.J. (1980) A reanalysis of the factors influencing basal metabolic rate in normal adults. *American Journal of Clinical Nutrition*, 33: 2372-74.

Cunningham, J.J. (1982) Body composition and resting metabolic rate: the myth of femenine metabolism. *American Journal of Clinical Nutrition*, 36, 721-726.

Dauncey, M.J. (1981) Influence of mild cold on 24h energy expenditure, resting metabolism and diet induced thermogenesis. *British Journal of Nutrition*, **45**, 257-267.

Delawaide, P.A. & Crenier, E.J. (1973) Body potassium as related to lean body mass measured by total water determination and by anthropometric method. *Human Biology*, 45:3, 509-526.

von Döbeln, W (1956) Human Standard and maximal metabolic rate in relation to fat free body mass. *Acta Physiologica Scandinavia* **37**, suppt. 126.

Deurenberg, P., van der Kooy, K., Hulshof, T., & Evers, P. (1989) Body mass index as a measure of body fatness in the elderly. *European Journal of Clinical Nutrition*, **43**, 231-236.

Doré, C., Hesp, R., Wilkins, D. & Garrow, J.S., (1982) Energy requirements of obese patients after masive weight loss. *Human Nutrition: Clinical Nutrition.*, **36C**, 41-48.

Drumond, S. (1988) Seasonal variations in energy balance in rural Indian women. *PhD Thesis, University of Glasgow*, UK.

Dubois,D. & Dubois E.F., (1916) Clinical Calorimetry. A formula to estimate the approximate surface area if height and weight be known. *Arch. Interal Medicine*, **17**, 863-871.

Durnin, J.V.G.A. & Taylor, A. (1960) Replicability of measurements of density of the human body as determined by underwater weighing. *Journal of Applied Physiology*, **15**, 142-144.

Durnin, J.V.G.A. & Rahaman, M.M. (1967) The assessment of the amount of fat in the human body from measurements of skinfold thickness. *British Journal of Nutrition*, **21**, 681-689.

Durnin, J.V.G.A. & Womersley, J. (1971) The measurement of obesity and some comments on its treatment by fenfluoramine, with special reference to changes in metabolic rate and body composition. *South African Medical Journal*, **45**, suppl. 42-43.

Durnin, J.V.G.A. & Womersley, J. (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, **32**, 77-97.

Durnin, J.V.G.A. & Satwanti (1982) Variations in the assessment of the fat content of the human body due to experimental technique in measuring body density. *Annal of Human Biology*, 9:3, 221-225.

Durnin, J.V.G.A., McKay F.C. & Webster, C.I. (1985a) A new method for assessing fatness and desirable weight, for use in the armed services. *Technical report. University of Glasgow.*

Durnin, J.V.G.A., McKillop F.M., Grant, S., & Fitzgerald, G. (1985b) Is nutritional status endangered by virtually no extra intake during pregnancy?. *The Lancet*, 823-825.

Durnin, J.V.G.A. & McKillop, F.M. (1988) The maternal fat component in the weight gain of pregnant women. *Proceedings of the Nutrition Society*, **47**, 6A.

Edholm, O.G., Fletcher, J.G., Widdowson, E.M. & McCance, R.A., (1955) The energy expenditure of food intake of individuial men, *British Journal of Nutrition*, **9**:286.

Edwards, D.A.W. (1950) Observation on the distribution of subcutaneous fat. *Clinical Science*, **9**, 259-270.

Edwards, D.A.W. (1950) Differences in the distribution of subcutaneous fat with sex and maturity. *Clinical Science*, **10**, 305-315.

Espinosa, Z.M.T., Bourges, R.H. & Durnin, J.V.G.A. (1992) Bone mineral content measured by DEXA in Mexican young adults. Unpublished observations.

FAO/WHO/UNU (1985) Energy and Protein Requirements. Report of a joint FAO/WHO/UNU Expert Consultation. World Health Organization. *Technical Report Series* 724. WHO, Geneva, 1985.

Finer, N., Swan, P.C., & Mitchell, F.T. (1986) Metabolic rate after massive weight loss in human obesity. *Clinical Science*, **70**, 395-398.

Forbes, G.B. & Amirhakimi, G.H. (1970) Skinfold thickness and body fat in children. *Human Biology*, **42**, 401-418.

Forbes, R.M., Cooper, A.R., & Mitchell, H.H. (1953) The composition of the adult human body as determined from chemical analysis. *Journal of Biological Chemistry*, **203**, 359-366.

Forbes, G.B., Gallup, J. & Hursh, J.B. (1961) Estimation of total body fat from Potassium-40 content. *Science*, **133**, 101-102.

Forbes, G.B., Schulz,F., Cafarelli, C. & Amirhakimi, G.H. (1968) Effects of body size on potassium-40 measurements in the whole body counter (tilt chair technique). *Health physics*, 435-442.

Urinary creatinine excretion and lean body mass
Forbes, GB & Bruining (1976) ^ *American Journal of Clinical Nutrition*, 29, 1359-1366.

Forwood, M.R. & Burr D.B. (1993) Physical activity and bone mass: exercises in futility?. *Bone and Mineral*, **21**, 89-112.

Garn, S., Leonard, W. & Hawthorne, V. (1987) Reply to letter from Miccozzi and Albanes. *American Journal of Clinical Nutrition*, 46; 377.

Garrow, J.S. (1982) New approaches to body composition. *American Journal of Clinical Nutrition*, 35, 1152-1158.

Gersh, I., Hawkinson, G.E., & Rathbun, E.N. (1944) Tissue and vascular bubbles after decompression from high pressure atmospheres - correlates of specific gravity with morfological changes. *Journal Cell and Comp. Physiology*, **24**, 35-61.

Gold, A.J., Zornitzer, A. & Samueloff, S. (1969) Influence of season and heat on energy expenditure during rest and exercise, *Journal of Applied Physiology*, **27**, 9.

Grande, F., Anderson, J.T., & Keys, A., (1958) Changes in basal metabolism rate in man in semistarvation and refeeding. *Journal of Applied Physiology*, **12**:230.

Halliday, D. (1985) Developments in total body water measurement. In *Human Body Composition and Fat distribution*, ed. Norgan N.G. Euro Nut report 8, 49-63. Stichting Voeding Nederland, Wageningen.

Harris, J.A. & Benedict, F.G., (1919) A biometric study of basal metabolism in man. *Carnegie Institute of Washington, Publication* No. 279:1.

Henry, C.J.K., Piggott, D.G., Rees, D.G., Priestly, L.,& Sykes, B. (1990). Basal metabolic rate in monozygotic and dizygotic twins. *European Journal of Clinical Nutrition* 44, 717-723.

Heusner, A.A. (1985) Body size and energy metabolism. *Annual Review of Nutrition*, **5**, 267-93.

Jackson, A.S. & Pollok, M.L. (1976) Factor analysis and multivariate scaling of anthropometric variables for the assessment of body composition. *Medical Science of Sports*, **8**, 196-203.

Jackson, A.S. & Pollok, M.L. (1977) Prediction accuracy of body density, lean body weight, and total volume equations. *Med. Science Sports*, **9**, 197-201.

James, W.P.T., Davis, H.L., Bailies, J., & Dauncey, M.J. (1978) Elevated metabolic rate in obesity. *The Lancet*, **2**, 1122-1125.

James, W.P.T., Dauncey, M.J., & Davis, H.L. (1978) Elevated metabolic rate in obesity. *The Lancet*, **2**, 472 (letter).

Jequeir, E. (1987) Energy utilization in human obesity. *Annals of the N.Y. Academy Science*, **499**, 73-83.

Jones P.R.M. Satwanti. (1982) Relationship of body density and lean body mass to body measurements: application to Indian soldiers of relationships developed for people of european descent. *Annals of Human Biology*, **9**:4, 355-362.

Jung, R.T., Shetty, P.S., & James W.P.T. (1980) The effect of ß-adrenergic blockade on metabolic rate and peripheral thyroid metabolism in obesity. *European Journal of Clinical Investigation*, **10**, 179-182.

Katch, V. (1973) Use of the oxygen/body weight ratio in correlational analyses: spurious correlations and statistical considerations. *Med. Science Sports*, **5**:4, 253-257.

Katch, V.& Katch F.I. (1974) Use of weight-adjusted oxygen uptake scores that avoid spurious correlations. *Research Quarterly*, **45**:4, 447-451.

Keys, A. & Brozek, J. (1953) Body fat in adult man. *Physiological Reviews*, **33**, 245-325.

Keys, A., Taylor, H.L., & Grande, F. (1973) Basal metabolism and age of adult man. *Metabolism*, **22**, 579-587.

Kleiber, M. (1932) Body size and metabolism. *Hilgardia*, 6:315.

Kleiber, M. (1947) Body size and metabolic rate. *Physiological Reviews*, 27, 511-541.

Kleiber, M. (1950) Physiological meaning of regression equations. *Journal of Applied Physiology*, 2:417-423.

Kleiber, M. (1961) The fire of life: an introduction to animal energetics. In *Nutrition. An integrated approach*, ed. Pike, R.L. & Brown, M.L. (1975), 2nd. ed., John Wiley & Sons, Inc. New York.

Kleinbaum, D.G. & Kupper, L.L. (1978) Comparing two straight-line regression models. In *Applied regression analysis and other multivariate methods*. Duxbury Press. Boston, Massachusetts. Ch. 8, pp 95-108.

Koong, L.J. & Ferrel, C.L. (1990) Effects of short term nutritional manipulation on organ size and fasting heat production. *European Journal of Clinical Nutrition*, **44** (Suppl 1), 73-77.

Krall, E.A. & Dawson-Hughes, B. (1993) Walking, bone mineral density and bone loss. *Journal of Bone Mineral Research*, **8** (suppl. 1): S150.

Kramer, M.S. & Feinstein, A.R. (1981) Clinical biostatistics LIV. The biostatistics of concordance. *Clinical Pharmacol Therapy*, **29**, 111-123.

Lawrence, M., Thongprasert, K. & Durnin, J.V.G.A. (1988) Between group differences in basal metabolic rates: an analysis of data collected in Scotland, The Gambia and Thailand. *European Journal of Clinical Nutrition*, **42**, 877-891.

Lawrence M. (1990) Estimation of body fat from total body water - methodological considerations. Institute of Physiology. University of Glasgow.

Lawrence M., McKillop, F.M. & Durnin J.V.G.A. (1991) Women who gain more fat during pregnancy may not have bigger babies: implications for recommended weight during pregnancy. *British Journal of Obstetrics & Gynaecology*, **98**, 254-259.

Lee, J., Kolonel, L. & Hinds, W. (1981) Relative merits of the weight-corrected-for-height indices. *American Journal of Clinical Nutrition*, **34**, 2521-2529.

Lohman, T.G. (1981) Skinfolds and body density and their relation to body fatness: A review. *Human Biololy*, **53:2**, 181-225.

Lowis, C. (1990) Studies on the variability of human basal metabolic rate. PhD Thesis. Institute of Physiology, University of Glasgow.

Lukaski, H. (1987) Methods for the assessment of human body composition: traditional and new. *American Journal of Clinical Nutrition*, **46**, 537-556.

Lye M. (1981) Distribution of body potassium in healthy elderly subjects. *Gerontology*, **27**, 286-292.

MacDonald F. (1986) Quetelet index as indicator of obesity. *The Lancet*, 1043.

Martin, D. & Notelovitz, M. (1993) Effects of aerobic training on bone mineral density of postmenopausal women. *Journal of Bone & Mineral Research*, **8**, 931-936.

Mason, E.D. & Jacob, M. (1972) Variations in basal metabolic rate responses to changes between tropical and temperate climates, *Human Biology*, **44**, 141-172.

McLaren D. (1987) Letters to the Editor: Three limitations of the body mass index. *American Journal of Clinical Nutrition*, **46**, 121.

McNeil, G., Rivers, J.P.W., Payne, P.R., de Britto. J.J., & Abel, R. (1987) Basal metabolic rate of Indian men: no evidence of metabolic adaptation to low plane of nutrition. *Human Nutrition: Clinical Nutrition*, **41C**, 473-484.

Méndez J. & Keys A. (1960) Density composition of mammalian muscle. *Metabolism*, 9, 184-188.

Messinger, W.J. & Steele. J.M. (1960) Relationship of body specific gravity to body fat and water content. *Proceedings of the Society of Experimental Biological Medicine*, 70, 316-318.

Micozzi, M. & Albanes, D. (1987) Letters to the Editor: Three limitations of the body mass index. *American Journal of Clinical Nutrition,* **46**, 376.

Mitchel, H.H. (1962) Comparative Nutrition of Man and Domestic Animals. Academic Press, N.Y. In *Nutrition. An integrated approach,* ed. Pike, R.L. & Brown, M.L. (1975). 2nd. ed. New York, John Wiley & Sons, Inc.

Moore, F.D., Olsen, K.O., McMurrey, J.D., Parker, H.V., Ball, M.R. & Boyden, C.M. (1963) *The body cell mass and its supporting environment.* Philadelphia, Pa.: Saunders.

Myhre L.G. & Kessler, W.V. (1966) Body density and potassium 40 measurements of body composition as related to age. *Journal of Applied Physiology,* **21**:4, 1251-1255.

Nevill,A., Ramsbottm, R. & Williams, C. (1992) Scaling physiological measurements for individuals of different body size. *European Journal of Applied Physiology,* **65**, 110-117.

Norgan, N.G. & Durnin, J.V.G.A., (1980) The effect of 6 weeks of overfeeding on the body weight, body composition and energy metabolism of youn men. *American Journal of Clinical Nutrition,* **33**, 978-988.

Novak, L.P. (1972) Aging, total body potassium, fat-free mass, and cell mass in males and females between ages 18 and 85 years. *Journal of Gerontology,* **27**:4, 438-443.

Owen, O.E., Holup, J.L., D'Alessio, D.A., Craig, E.S., Polansky, M., Smalley, K.J., Kavle, E.C., Bushman, M.C., Owen, L.R., Mozzoli, M.A., Kendrik, Z.V. & Boden, G.H. (1987) A reappraisal of the caloric requirements of men. *American Journal of Clinical Nutrition,* **46**, 875-885.

Pascale, L.R., Grossman, M.I., Sloan, H.S. & Frankel, T. (1956) Correlations between thickness of skinfolds and body density on 88 soldiers. *Human Biology,* **28**, 165-176.

Passmore, R. & Durnin, J.V.G.A. (1967) *Energy, work and leisure.* London: Heinemann Educational Books.

Pierson, R.N., Lin, D.H. & Phillips, R.A. (1974) Total body potassium in health: effects of age, height, and fat. *American Journal of Physiology*, **226**:1, 206-212.

Pierson R.N., Colt, J.W. & Neuman P. (1982) Body composition measurements in normal man: the potassium, sodium, sulfate and tritium spaces in 58 adults. *Journal of Chronic Disease*, **34**, 419-428.

Pike, R.L. & Brown, M.L. (1975). *Nutrition. An integrated approach*, 2nd. ed. New York. John Wiley & Sons, Inc.

Pitts, G.C. & Bullard, T.R. (1968) Some interspecific aspects of body composition in man. In *Body composition in animals and man*, ed. Brozek, J. Publ. No. 1598. Washington, D.C.: National Academy of Sciences.

Prentice, T.C., Siri, W.E., Berlin, N.E., & Joiner, E.E. (1952) *Journal of Clinical Investigation*, **31**, 412.

Ravussin, E., Burnand, B., Schutz, Y. & Jequier, E. (1982) Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese and control subjects. *American Journal of Clinical Nutrition*, **35**, 566-573.

Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L., & Bogardus, C. (1986) Determinants of 24-hour energy expenditure in man. *Journal of Clinical Investigation*, **78**, 1568-1578.

Ravussin, E., Lillioja, S., Knowler, W.C., Christin, L., Freymond, D., Abbot, W.G.H., & Bogardus, C. (1988) Reduced rate of energy expenditure as a risk factor for body weight gain. *New England Journal of Medicine*, **318**, 467-472.

Reeds, P.J., Fuler, M.F. & Nicholson, B.A. (1985) Metabolic basis of energy expenditure with particular reference to protein. In *Substrate and energy metabolism in man*, ed. J.S. Garrow & D. Halliday, pp 46-57. London, J. Libbey.

Ross, W.D., Crawford, S.M., Ward, K.R., Bailey, D.A. & Mirwald, R.M. (1988) Relationship of the body mass index with skinfolds, girhts and bone breaths in Canadian men and women aged 20-70 years. *American Journal of Physical Anthropology*, **77**, 169-173.

Schmidt-Nielsen K. (1984) Scaling: why is animal size so important?. Cambidge University Press, Cambridge. In Scaling physiological measurements for individuals of different body size. Nevill, A., Ramsbottm, R. & Williams, C. (1992). *European Journal of Applied Physiology*, **65**, 110-117.

Schofield, C. (1985) Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition: Clinical Nutrition*, **39C**, Supl. 1,5-41.

Scholoerb, P.R., Friis-Hansen, B.J., Edelman, I.S. Solomon, A.K. & Moore, F.D. (1950) *Journal of Clinical Investigation*, **29**, 1296.

Schutz, Y., Acheson, K., Bessard, T. & Jequier, E. (1982) Effect of a 7-day carbohydrate (CHO) hyperalimentation on energy metabolism in healthy individuals. *Clinical Nutrition* **1**, Suppl. F100.

Shetty, P.S., James, W.P.T. & Jung, R.T. (1979) The effect of catecholamine replacement with levodopa on the metabolic response to semistarvation. *The Lancet*, **1**, 77-79.

Shukla, K.K., Ellis K.J., Dombrowski C.S. & Cohn S.H. (1973) Physiological variation of total-body potassium in man. *American Journal of Physiology*, **224**:2, 271-274.

Sims, E.A.H. (1976) Experimental obesity, dietary induced thermogenesis and their clinical implications. *Clinical Endocrinology Metabolism*, **5**, 377-395.

Sinning, W. (1978) Anthropometric estimation of body density, fat, and lean body weight in women gymnasts. *Medicine and Science in Sports*, **10**:4, 243-249.

Siri, W.B. (1956) The gross composition of the human body. In *Advances in Biological and Medical Physics*, vol. 4, ed. C.A. Tobias & J.H. Lawrence, pp 239-280. New York, Academic Press.

Siri, W.B. (1961) Body composition from fluid spaces and density. Analysis of methods. In *Techniques for measuring body composition*. pp 223-244. National Acad. Sci. -National Res. Council, Washington, D.C.

Sjöström L., Kvist H., Cederblad A. & Thlen U. (1986) Determination of total adipose tissue and body fat in women by computed tomography, ^{40}K, and tritium. *American Journal of Physiology*, **250** (Endocrinology Metabolism 13): E736-E745.

Skerlj,B., Brozek,J., & Hunt, E.E. (1953) Subcutaneous fat and age changes in body build and body form in women. *American Journal of Physical Anthropology*, n.s., **11**, 277-600.

Smalley, K., Knerr, A., Kendrick, Z., Colliver, J. & Owen, O. (1990) Reassessment of body mass indices. *American Journal of Clinical Nutrition*, **52**, 405-408.

Smith, D.A., Anderson, J.B., Shimmins, J., Speirs, C.F. & Barnett, E. (1969) Changes in metacarpal mineral content and density in normal male and female subjects with age. *Clinical Radiology*, **20**, 23-31.

Sorenson, J.A., Mazess, R.B., Smith, E.L., Clark, J.A. & Cameron, J.R. (1968) Bone mineral content and bone diameter vs. age in the radius and humerus of normal subjects. Bone Mineral and body composition progress report - 1968 University of Wisconsin.

Spady, D.W., Payne, P.R., Picou, D., & Waterlow, J.C. (1976) Energy balance during recovery from malnutrition. *American Journal of Clinical Nutrition*, **29**, 1073-1078.

Surveyor, I. & Hughes D. (1968) Discrepancies between whole body potassium content and exchangeable potassium. *Journal Laboratory & Clinical Medicine*, **71**:3, 464-472.

Talso, P.J., C.E. Miller, A.J. Carballo & I. Vasquez (1960) Exchangeable potassium as a parameter of body composition. *Metabolism*, 9, 456-471.

Tanner, J.M. (1949) Fallacy of per weight and per-surface area standards, and their spurious correlation. *Journal of Applied Physiology*, 2, 1-15.

Trotter, M., Broman, G.E. & Peterson, R.R. (1959) Density of cervical vertebrae and comparison with density of other bones. *American Journal of Physical Anthropology*, **17** n.s., 19-26.

Valencia, M.E., Moya. S. Y., McNeil, G & Haggarty, P.. (1994) Basal metabolic rate and body fatness of adult men in northern Mexico. *European Journal of Clinical Nutrition*, **48**, 205-211.

Webb, P. (1981) Energy expenditure and FFM in men and women. *American Journal of Clinical Nutrition*, **34**, 1816-1826.

Weir, J.B. de V (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *Journal of Physiology* (Lond)., **109**:1.

Widdowson, E.M. (1968) Biological implications in body composition. In *Body composition in animals and man*, ed. Brozek, J. Publ. No. 1598. Washington, D.C., National Academy of Sciences.

Wilmore, J.H. & Behnke, A.R. (1968) Predictability of lean body weight through anthropometric assessment in college men. *Journal of Applied Physiology*, **25**, 349-355.

Wilmore, J.H. & Behnke, A.R. (1969) An anthropometric estimation of body density and lean body weight in young men. *Journal of Applied Physiology*, **27**, 25-31.

Winter, E.M., Brookes, F.B.C. & Hamley E.J. (1991) Maximal exercise performance and lean leg volume in men and women. *Journal of Sports Science*, **9**, 3-13.

Witters, R.T., Whittingham, N.O., Norton, K.I., La Forgia, J., Ellis, M.W. & Crocket A. (1987) Relative body fat and anthropometric prediction of body density of female athletes. *European Journal of Applied Physiology*, **56**, 169-180.

Witters, R.T., Norton, K.I., Craig N.P., Hartland, M.C., & Venables W. (1987) The relative body fat and anthropometric prediction of body density of South Australian females aged 17-35 years. *European Journal of Applied Physiology*, **56**, 181-190.

Witters, R.T., Craig, N.P., Bourdon, P.C., & Norton, K.I. (1987) Relative body fat and anthropometric prediction of body density of male athletes. *European Journal of Applied Physiology*, **56**, 191-200.

Woo, R., Daniels-Kush, R. & Horton, E.S. (1985) Regulation of energy balance. *Annual Review of Nutrition*, 5, 411-433.

Womersley, J., Boddy, K., King P. C. & Durnin, J.V.G.A. (1972) A comparison of the fat-free mass of young adults by anthropometry, body density and total body potassium content. *Clinical Science.*, **43**, 469-475.

Womersley, J. & Durnin, J.V.G.A. (1973) An experimental study on variability of measurements of skinfold thickness on young adults. *Human Biology*, **45**:2, 281-292.

Womersley, J. (1974) Fat and the Fat-Free Mass in Man. PhD thesis. Institute of Physiology, University of Glasgow.

Womersley, J., Durnin, JVGA, Boddy, K. & Mahaffy M. (1976) Influence of muscular development, obesity, and age on the fat- free mass of adults. *Journal of Applied Physiology*, **41**:2, 223-229.

Womersley, J. & Durnin, J.V.G.A. (1977) A comparison of the skinfold method with extent to 'overweight' and various weight-height relationships in the assessment of obesity. *British Journal of Nutrition*, **38**, 271-284.

Yeh, J.K., Aloia, J.F., Chen M.M., Tierney J.M., & Sprintz, S. (1993) Influence of exercise on cancellous bone of the aged female rat. *Journal of Bone and Mineral Research*, **8**, 1117-1125.

Young, C.M., Blondin, J., Tensuan, R. & Fryer, J.H. (1963) Body composition of 'older' women. *Journal of the American Dietetic Association.*, **43**, 344-348.

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